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STUDIES IN THE PHYSIOLOGY OF THE FUNGI

XII. Physiological Specialization in Rhizoctonia Solani Kühn

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Introduction

The study of the diseases induced by *Rhizoctonia* has been undertaken by many investigators in different countries since the first report of the occurrence of this organism in 1728, in France. Nevertheless, the occurrence of this fungus on such a diversity of host plants and the possible existence of distinct forms or races within the species suggest that there are many phases of the subject still requiring extensive investigation.

The chief object of the present investigation was to make a comparative study of such strains of *Rhizoctonia Solani* Kühn as could be obtained from different disease types of the same host.

It is a well-known fact that a culturable fungus may exhibit considerable differences in morphological characteristics and in physiological behavior under the influence of changes in the culture media or other environmental conditions. On the other hand, within the species there may exist forms or races which in no sense represent the effects of simple environmental factors. These races show more or less distinctive and constant morphological and physiological characteristics under any particular

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set of conditions, and these characteristics are inherited; and taken as a whole, they differ from those of any other race under similar conditions.

For the determination of the species, forms, or races, therefore, it is necessary to take into consideration all of the factors which have been referred to, and a comparison should be attempted only after careful observation of physiological and morphological characters, accompanied by extensive inoculation experiments. It is necessary to make comparative studies between the original and reisolated strains, the latter being obtained from the plants used in the inoculation experiments.

LITERATURE REVIEW

An adequate review of the literature concerning the diseases caused by *Rhizoctonia* may be had by recourse to the papers of Duggar ('15) and Peltier ('16). Especially is the early European literature extensively reviewed in the paper first mentioned. Therefore I will permit myself only a brief review of some of the more important papers closely connected with my present investigation.

Since the first description of *Rhizoctonia* by DeCandolle in 1818, many species have been described by different authors. The Tulasne brothers, who gave the most complete mycological account of the genus, classified all the forms then known as a single species, *Rhizoctonia violacea* Tul., reducing all other names to synonyms. Later Kühn described a new species on potato. This species, at least, he clearly distinguished from the above, and he named it *R. Solani* Kühn.

In the United States, since the first report of the disease of alfalfa mentioned by Webber, who considered it as identical with *Rhizoctonia Medicaginis* DC., many papers have been presented, such as those of Pammel ('91), discussing its occurrence on beets, and of Atkinson ('92), reporting a sterile fungus on cotton seedlings, and later work showed its occurrence on a number of other kinds of seedlings. Nevertheless, credit for the comprehensive account of *Rhizoctonia* in America should be given to Duggar ('99). He studied different types of plant diseases due to a common *Rhizoctonia* and showed that the beet fungus and carnation fungus were identical, although the special

affinities of these could not be given with certainty. Subsequently, Duggar and Stewart ('01) added a large number of hosts subject to *Rhizoctonia* attack and gave proof that the organism, or forms of the organism, exhibited morphologically and in culture the characters of the beet rot and damping-off fungus. Later, Duggar ('15), after a most elaborate study of the common *Rhizoctonia* (designated *R. Solani* Kühn), made the following statement: "In the different strains which have been studied, originating from different hosts, certain minor modifications of the general habit of the fungus in culture have been observed. But these have not seemed to be sufficient to be considered of specific importance, except in the case of the form on the rhubarb." Further, he said: "The subject needs further investigation, but in general it is felt that these differences are such as might be due to permanent differences in the pathological strains, on the one hand, or may be regarded as temporary differences due to the recent environment, on the other." Furthermore, in that paper he discussed more extensively the relationship between the violet root felt fungus described by Tulasne as *R. violacea* and the common *Rhizoctonia*, and gave some of the important and easily observed contrasting features as usually found in these forms. He proposed that the first-named fungus should be designated as *R. Crocorum* (Pers.) DC., inasmuch as the more appropriate descriptive name, *R. violacea* Tul., does not, unfortunately, conform to the international rules of nomenclature.

At about the same time Peltier ('16), after a prolonged study of the common *Rhizoctonia* occurring in America, arrived at the conclusion that all strains studied by him could be included under one species, *Rhizoctonia Solani* Kühn, for no marked specialization was noted in any of the strains. His argument is as follows: "From these inoculation experiments with a large number of different types of plants we must conclude that all the strains studied, which were obtained from a wide range of hosts of diverse geographical origin, can attack the same species of plant and produce the same characteristic symptoms. No marked specialization was noted in any of the strains." From the culture experiments he observed that the growth of the strains was very variable, those from the same host often producing a dif-

ferent type of growth even on the same media, and that the differences in various cultural characters which were shown by strains from unlike hosts were no greater than the differences which might be manifested by two different strains isolated from the same host, or by the same strain after being kept for different intervals of time in artificial cultures. He further stated that measurements of mycelial and sclerotial cells of the fungus showed large variations, not only between strains from different hosts but also between different strains from the same host; therefore no standard could be determined upon as a means of distinguishing the different strains. Duggar ('16) concluded that the common seed-bed fungus in Germany and in France was identical with the damping-off fungus which had been frequently studied in the United States by Atkinson. Rosenbaum and Shapovalov ('17) studied *Rhizoctonia* diseases of the potato in Maine and proposed a new strain of *Rhizoctonia Solani* Kühn based on the idea that the new strain might be distinguished from the more common *Rhizoctonia* (1) by the more pronounced lesions produced when inoculated into injured stems or tubers; (2) by the reaction, growth, and character of sclerotia on definite media; and (3) by morphological characters, especially by the measurement of the short sclerotial cells of the mycelium; and lastly by the diameter of germ tubes.

Ramsey ('17), working on the form of potato tuber disease produced by these fungi, noticed two important phases of the injury: In one of these the external appearance somewhat resembles scab and extends a dry core into the flesh of the tuber; in another the shrinkage of tissues forms a pit or canal in the center of the infected area, frequently suggesting wire worm injury. Concerning the form of the causal fungus, however, no adequate description was given.

In the same year Matz ('17) described a new species of *Rhizoctonia* on figs. According to him the sclerotia of this species are quite different from those of the common forms. Therefore he proposed the name *Rhizoctonia microsclerotia* for this species.

Concerning the studies on specialization of forms in the species here discussed, absolutely nothing has been reported, although the literature dealing with the specialization of other fungi is rather extensive; especially has the work been elaborate in

regard to the rusts and powdery mildews. A brief review of the important studies will not be superfluous in this connection.

Magnus might be considered one of the early investigators in this line. He suggested that a particular biologic form might, by constant association with one host, change its physiological capabilities to such an extent as to develop a new race. Eriksson ('94), in his cross-inoculation experiments with *Puccinia graminis*, observed the evidence of biologic specialization and noticed that the form upon one host species was not always indentical with the form upon another. Dietel ('99) also noticed that a rust fungus which had been capable of attacking a number of plants acquired by long association with one species of host somewhat weakened capabilities of attacking other forms. Ward ('02), in his study on the relations between host and parasite in the bromes and their brown rust, suggested that each specialized form of *Puccinia* might during the lapse of time actually become a distinct species. Eriksson ('02) further stated, in a subsequent paper, that the trend of specialization might be different in isolated localities. Furthermore, Ward ('03), in his excellent work concerning the occurrence of a "bridging species," indicated that some forms of bromes might act as "bridging species" in enabling the rust to pass indirectly from one group of bromes to another, although direct transfer was impossible.

Salmon ('04) also observed a similar phenomenon in *Erysiphe graminis*. The same author in his later work showed that the virulence of *Erysiphe graminis* might be changed by certain cultural conditions. By injuring leaves and subjecting plants to heat, etc., the author was able to infect forms which seemed normally immune. Reed ('02, '16), in his diverse cross-inoculation experiments with *Erysiphe graminis*, noticed some considerable variation in susceptibility among the species and varieties of *Triticum*, *Hordeum*, *Avena*, and *Secale*, and defined the existence of biologic specialization. Shear and Wood ('13) stated that *Glomerella cingulata* was exceedingly variable in all its characters so far as they had been studied, although the cause of this variability was not clear. Further, they noticed that no constant or definite relation had been established between the environmental conditions and the most important variations observed. They said: "In any case the evidence accumulated

by others as well as by the writers appears sufficient to justify the conclusion that many of the variations observed and reported here are not entirely due to any effect of simple environmental factors." Further, they said: "The work of Jennings ('11) with Paramecium and that of Barber ('07), Will ('90), Beijerinck ('97) and Hansen ('00) on yeasts, as well as that of other authors cited by Pringsheim ('10), demonstrate at least one thing, and that is the actual existence of rather distinct races or strains within species. These races possess more or less distinctive and constant morphological or physiological characteristics which are generally inherited by their progeny and are apparently not primarily dependent upon environmental conditions." Referring to the common rust of wheat, Stakman ('14) says: "On the most resistant varieties, such as Khapli, the spores are often small in size and sometimes abortive." From a study of biologic specialization in the genus *Septoria* Beach ('19) observed that certain species are differentiated into biologic forms. According to him, disease characters as manifested by the host and some morphological characters of certain species of *Septoria* vary with the host and with environmental conditions and are therefore unreliable in taxonomy.

Concerning the specialization of rusts, Klebahn ('17) expressed an opinion, which unfortunately I have been unable to see in the original, but from the abstract by Matouschek ('19) the following is suggestive of the position taken: "Die fluktuierenden Variationen und die Mutationen sind ja Veränderungen, die, wenn auch vielleicht von der Aussenwelt beeinflusst, aus dem inneren Wesen des lebenden Protoplasmas hervorzugehen scheinen—und diese spielen bei Entstehung der Formenunterschiede vielleicht eine grössere Rolle als bei der Ausbildung der biologischen Verschiedenheiten."

I should not neglect to mention also such studies as those of Stäger on *Claviceps*, of Diedicke on *Pleospora*, of Gilbert on *Plowrightia*, of Müller on *Rhytisma*, and of Hesler on *Sphaeropsis malorum*, etc.

SOURCE OF MATERIALS

All the strains obtained by me were first isolated by using potato agar, as this medium is very suitable for the mycelial

and sclerotial growth of the fungi studied. This medium also affords a convenient means of separating the various forms into the following major groups: (1) the strains which with growth blacken the agar; and (2) the strains which do not blacken agar.

THE STRAINS WHICH BLACKEN AGAR

P1, isolated from a badly affected stem of potato received from Prof. W. T. Horne, California, 1917.

P2, a culture obtained from a potato stem collected by me at Berkeley, California, 1917.

P3, origin similar to the preceding.

L1, a culture of this strain obtained from a very badly infected lettuce plant, greenhouse, Missouri Botanical Garden.

E1, a culture obtained from a diseased egg-plant (3 inches high), Berkeley, California.

H, obtained from Dr. S. M. Zeller, by whom it had been isolated from *Habenaria* sp. (and kept in an incubator for about 2 months).

NO PRONOUNCED, AND AT MOST SLIGHT, BLACKENING OF AGAR

P4 was isolated from sclerotia found on potato tuber obtained on the market, St. Louis, 1918.

P5, origin similar to the preceding.

P6, isolated from sclerotia on potato tuber obtained in the market, Berkeley, California, 1917.

B1, isolated from a brown lesion on the stem of white navy bean, given by Miss E. H. Smith, California, 1917. The plant was not badly infected, having comparatively healthy roots and bearing four pods.

L2, from stock culture in the laboratory of the Missouri Botanical Garden. This strain was isolated by one of the former graduate students, but the record of its habitat was not at hand.

B2, obtained from the brown lesion of a certain variety of navy beans collected by Dr. Duggar, 1919.

AGAR NOT BLACKENED

P7, isolated by me from a potato stem, Berkeley, California. The plant was not seriously affected, but all parts were considerably dried when found in the late part of autumn, 1917. The

bark of the plant, especially near the ground, was much injured and easily peeled from the stem. A net-work of fungous threads was abundantly seen on the inside of the bark.

D, obtained from a dahlia plant in the garden just mentioned. The plant was perfectly dry and the bark of the plant was very easily removed from the stem. To my great regret I lost the strain during the progress of this study, so that in the present paper I am unable to report any extended result with it. But from the preliminary experimental work done by me during my stay at the University of California, it seems safe to say that it is identical with P7.

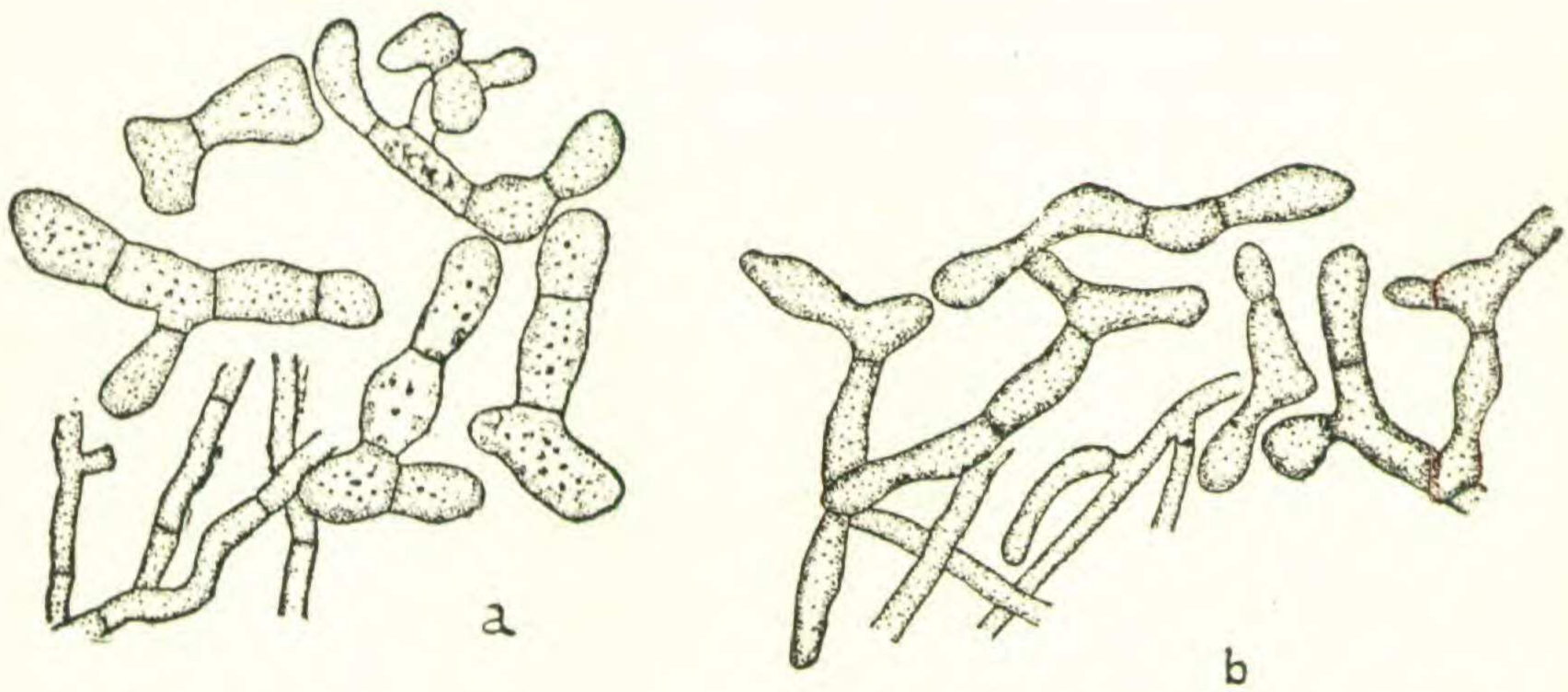

Fig. 1. *a*, sclerotial and hyphal cells of P1; *b*, sclerotial and hyphal cells of P4 (camera lucida drawings).

B3, isolated from the *Corticium* stage found on the stems of rather healthy lima beans grown in the Missouri Botanical Garden, 1918. The affected plants appeared practically healthy, having many pods. The *Corticium* stage was also observed on the pods, or leaves, and even on small areas of the soil adjoining the plants.

The account of *Corticium* as a perfect stage of *Rhizoctonia Solani* was first recorded by Rolfs ('03), and subsequently ('04) a more detailed description was published by the same author. The fruiting stage found by him was more or less related to *Corticium vagum* B. & C., but its apparent parasitic mode of life and the size and shape of spores were considered of sufficient importance to establish it as a new variety, and it was designated *Corticium vagum* B. & C. var. *Solani* Burt. However, Burt

('18) later identified the *Corticium* causing disease as the common *Corticium vagum* B. & C., reducing the variety to synonymy. My material was examined by Dr. Burt and determined to be identical with *Corticium vagum* B. & C. For the isolation of the fungus Rolfs suspended the fruiting layer over a Petri dish containing agar, covering stem and dish with a sterile bell jar. If, however, a fruiting stage is too young or too old, this method is unsatisfactory and the dilution method is preferable. The method applied by me consists in touching the hymenial layer with a sterile platinum needle which is immediately removed into sterile water before transfer to melted agar.

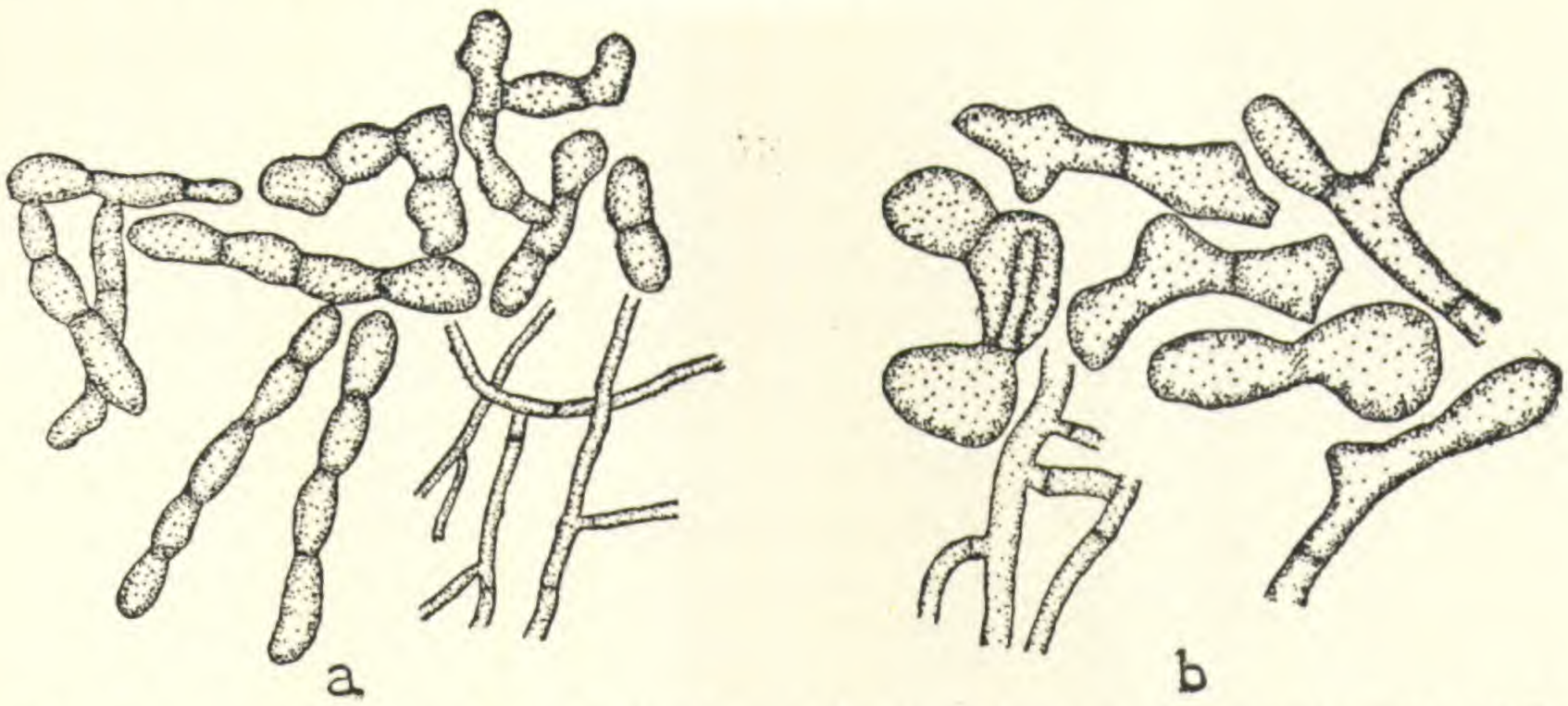

Fig. 2. *a*, sclerotial and hyphal cells of P7; *b*, sclerotial and hyphal cells of B1 (camera lucida drawings).

From the preliminary work it appeared that among the strains mentioned above P1, P2, P3, L1, and E1 are very closely related to each other in general morphological characters, in reaction on potato agar, in shape and color of sclerotia, in mycelial characters, etc. Likewise, the group of cultures P4, P5, and P6 are also apparently identical; while another group of similar forms includes B1, L2, and B3.

Therefore, for further extensive studies concerning the physiological specialization of the strains a single representative of each group is considered, namely, P1, P4, P7, B1, B3. In addition to these, H is also included. The last-named strain seemed identical with P1 in every respect, but for the reason that the strain was obtained from a different host and was of different geographical origin, it was decided to use it in the present experiments.

From the cultural experiments with a large number of different media I observed certain marked differences in morphological characteristics of the hyphae and sclerotia exhibited by the 6 strains, most of which are constant, showing no variation for any strain on the same medium and often none on different media. The most striking features shown by the strains will be summarized in the following table:

TABLE I

SHOWING THE MORPHOLOGICAL CHARACTERISTICS OF THE HYPHAE AND SCLEROTIA OF THE 6 STRAINS

	Diameter of hyphae (μ)	Size of sclerotial cells Extreme and average measurements (μ)	Color of sclerotia (on corn meal)*
P1	8–12	11–17 × 20–56 Average 12 × 40	Brick-red in young, cinnamon-brown or chestnut-brown in old
P4	8–13	17–23 × 26–48 Average 18 × 32	Chocolate in young, warm blackish brown in old
P7	3–6	8–13 × 15–28 Average 9 × 21	Clay-color to tawny olive
B1	8–14	12–34 × 29–54 Average 27 × 40	Mars brown or chocolate
H	7–11	8–20 × 19–48 Average 14 × 38	Hazel or brick-red in young, cinnamon-brown in old
B3	8–12	14–26 × 16–42 Average 25 × 36	Mars brown or darkish brown

* Color designations are in accordance with Ridgway's "Color Standards and Nomenclature."

As shown in the table, the sclerotia of B1 are strikingly large and roundish, while those of P7 are the smallest of all and very light in color. There is also a remarkable difference in diameter of hyphae between P7 and the other strains. In general, there is no striking difference between P1 and P4 and H, though there exist minor differences in color, size, and shape of sclerotia.

The shape of the sclerotia of the various strains will be more clearly demonstrated by the accompanying figures (figs. 1–3).

Influence of Temperature

Studies on the temperature relations of parasitic fungi are numerous. The experiments and observations alike demonstrate that the growth of a large number of organisms may be closely related to relatively narrow limits of thermal conditions. There are many diseases which develop and spread only during relatively cool seasons, while on the other hand, there are numerous cases which develop only during the hottest weather of summer.

Confining the discussion to parasitic species we shall pass without comment the important work of Wiesner, Tiraboschi, and Thiele. Schneider-Orelli ('12) reported temperature studies on different species or strains of *Gloeosporium fructigenum*, and found that the European form had lower optimum, maximum,

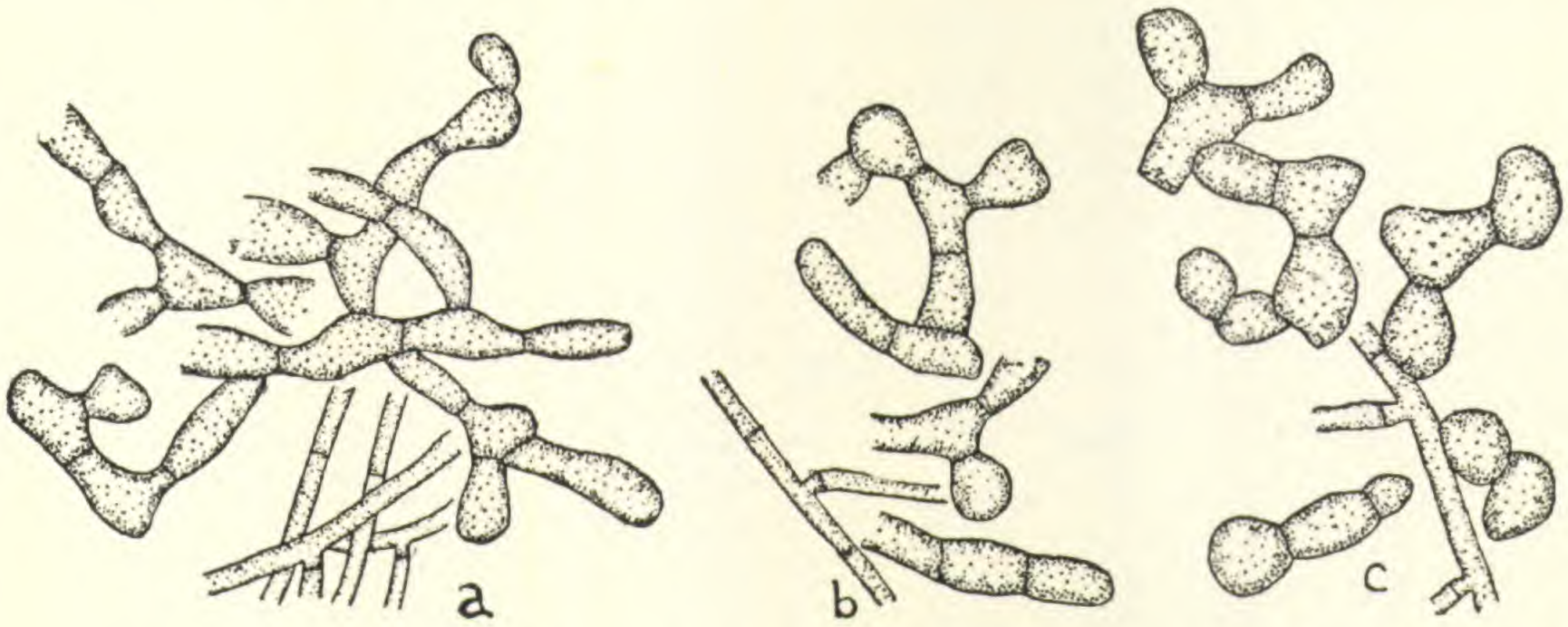

Fig. 3. *a*, sclerotial and hyphal cells of H; *b*, sclerotial and hyphal cells of B3; *c*, sclerotial and hyphal cells of the reisolated strain of B1 (camera lucida drawings).

and minimum temperature than the American form. At 5°C. the European form produced a colony 0.4 cm. in diameter in 12 days and a colony 3.7 cm. in diameter in 35 days, while the American form had made no growth at the end of 35 days.

Brooks and Cooley ('16) noticed that the temperature responses of the various fungi may be greatly modified by the food material upon which they are grown. *Fusarium radicicola* and *Glomerella cingulata* had a lower minimum temperature on corn meal agar than on fruit, and the early growth of species of

Alternaria, *Botrytis*, and *Penicillium*, etc., was much less inhibited on corn meal agar at low temperature than on the apple. In 1918 Hemmi, working with a large number of species of *Gloeosporium*, obtained from many different host plants, reported a similar relation concerning the maximum temperature of the strain isolated from loquat (*Eriobotrya japonica*). It is clear, therefore, that in any study of the temperature relations of fungi, it is very necessary to take into consideration all environmental factors, and comparative studies should always be performed under the same physiological conditions.

In cultures on potato agar it is observed that there is little difference in the rate of growth of the mycelium of B1 between 27 and 33° C., and the optimum temperature lies within this range, perhaps actually about 31° C. As no growth takes place at 13–15° C., the minimum is relatively higher. Above 33° C. the rate of growth of the same strain gradually decreases, and in most cases, according to my experiments, no new growth is secured at 44°C., showing that this temperature is about the maximum, or perhaps 42–44°C.

Almost the same results are obtained in the germination experiments with the sclerotia of B1. The germination of the sclerotia was studied in distilled water by the hanging-drop method at 34–36° C. to 22–24° C. After a few hours the sclerotia generally began to germinate and at the higher temperature a large number germinated, while at the lower a few only germinated, as shown in table II.

TABLE II

EXTENT OF GROWTH OF B1 AT DIFFERENT TEMPERATURES

Temperature	After 4 hours	After 8 hours	After 15 hours
34–36° C.	24μ	89μ	About 140μ
22–24° C.	No growth	38μ	About 95μ

In general, the three strains, P1, P4, and H, have about the same rate of growth at the different temperatures, although P4 differs slightly from the other 2 strains. The optimum temperature of these strains would seem to be about 24° C. At 14–15° C. there is still some new mycelial growth, so that the minimum

temperature of the strains is lower than that of B1. With regard to maximum temperature of those 3 strains named above, in most cases no growth is secured at 39–40° C., P4 in every case producing no new growth even at 38–39° C.

Concerning P7, there is no notable difference between 23 and 33° C., and at 14–16° C. there is still more or less growth. The minimum temperature is slightly lower than that of B1. At 37–40° C. the growth is much retarded, the maximum being somewhat lower than that of B1. The germination experiments show that the growth of P7, though slender, is more vigorous in all drop cultures than the remaining strains.

The growth of the mycelium of B3 on culture media is very slow, and at present I find that the strain grows better at a lower temperature (about 22° C.) than at a higher (about 35° C.).

The growth relations of the different strains at certain temperatures may be illustrated by giving in tabular form the results of one of the experiments—in which potato decoction was used as a medium.

TABLE III

GROWTH OF CERTAIN STRAINS AT DIFFERENT TEMPERATURES, PERIOD OF INCUBATION 3 WEEKS, DRY WEIGHT IN GRAMS

Strain	38° C.	22° C.	Room temperature*
P1	0.015	0.345	0.045
P4	Negl.	0.185	0.100
P7	0.015	0.120	0.010
B1	0.270	0.120	0.005
H	0.020	0.300	0.050

* This was 14–17° C. at night and 17–20° C. during the day.

Such relations resulting from other experiments are also shown graphically by fig. 4. In general, the growth during the first 2 days is very slight, so that in the figure there is given an average of the observations taken during the first 5 days.

Nutritive Metabolism with Special Reference to Enzymatic Activities

In general, the species of fungi exhibit a more or less marked specialization both qualitatively and quantitatively in respect

to secretion of enzymes. Many investigators have also shown that the formation of enzymes is more or less related to environmental factors. Although it has not yet been thoroughly established to what extent environmental factors are efficient in stimulating or retarding the formation of enzymes, brief reviews of some of this work should be presented in this connection.

Katz ('98) studied the regulatory secretion of amylase in *Aspergillus niger*, *Penicillium glaucum*, etc., and found that the effect of various other substances serving together with starch as a source of carbon is in general to inhibit the secretion of

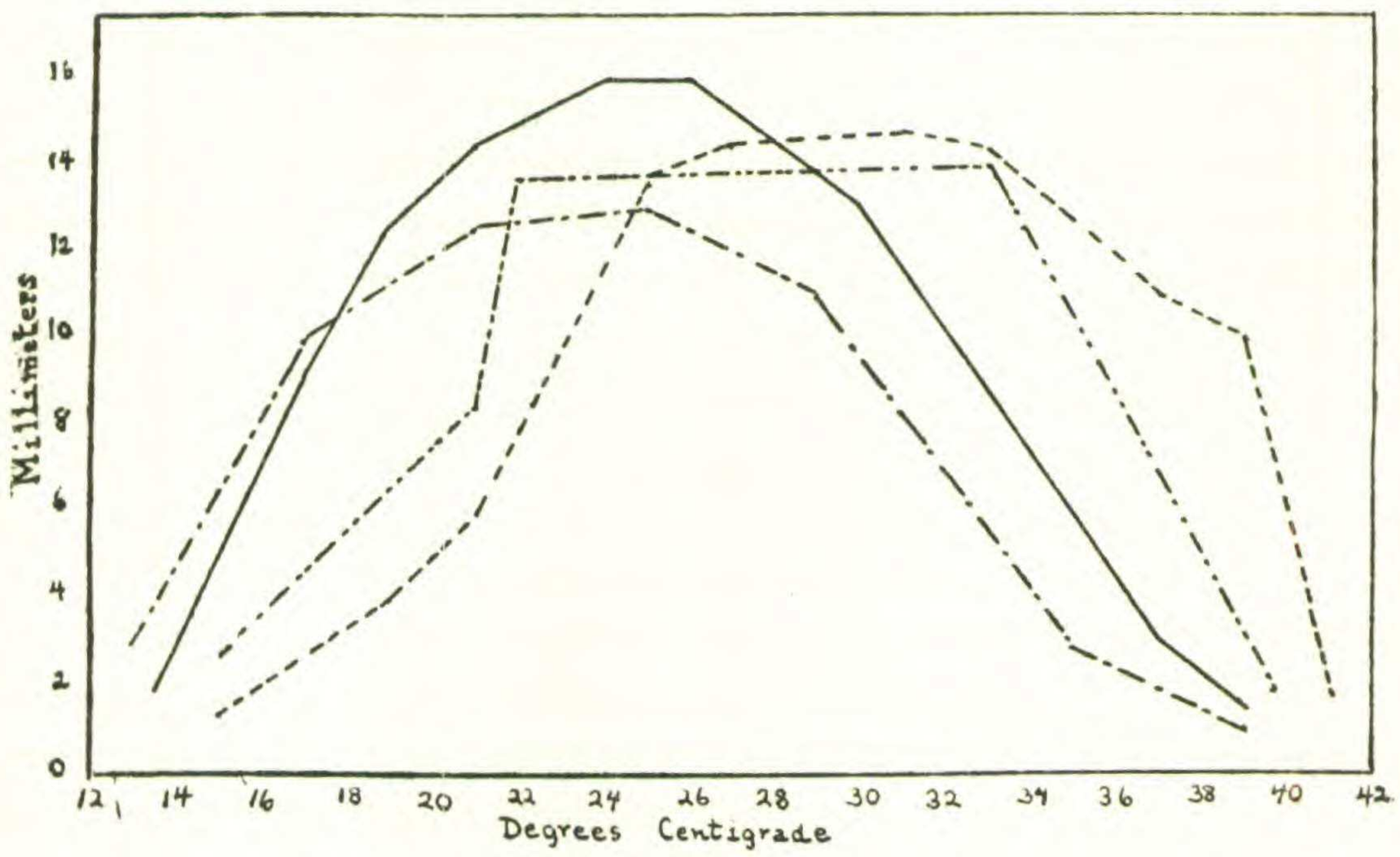

Fig. 4. Rate of growth on bean decoction agar. —— represents P1 and H; —·— P4; - - - - B1; —· ·—· · P7.

amylase, while the presence of starch alone in the culture medium stimulates the fungus to form a large amount of amylase. Herissey ('99) studied the appearance of emulsin in *Aspergillus niger* on Raulin's solution and found that the enzyme was observed only after 48 hours. If 3 to 4 times as much ammonium nitrate as usual were used and this amount replaced at the end of every 24 hours, such cultures grew for a month without sporulation and at the same time yielded no enzyme capable of hydrolyzing amygdalin.

Went ('01), in his study of *Monilia sitophila*, reported that at least 10 enzymes were formed by this fungus and he separated

these enzymes into 3 groups: (1) diastase, etc., which is found at least in small quantity when the fungus is grown on any medium; (2) maltoglucase, formed only when both carbohydrate and a nitrogen-containing salt are present; and (3), those that are formed only when certain substances chemically allied to the substratum are present; thus trehalase is formed when the culture medium contains trehalose.

Dox ('10) believed "that enzyms not normally formed by the organism in demonstrable quantities" could not be developed by special nutrition and that the effect of a particular substratum "is, therefore, not to develop an entirely new enzym, but to stimulate the production of the corresponding enzym." Roselli ('11), using *Aspergillus niger*, found that equal amounts of various carbohydrates did not affect the amount of inulase secreted materially, but the amount in the culture medium increased rapidly with age. Kylin ('14) could not find any evidence of qualitative enzyme regulation except in the case of tannase formation by *Aspergillus niger* and *Penicillium* sp., which is conditioned by the presence of tannic or gallic acid in the culture medium. Quantitative regulation, however, was found to be pronounced, and greater in the case of *Penicillium* than in *Aspergillus*.

In 1918 Young studied inulase formation in *Aspergillus niger* and concluded that under all conditions studied inulase was produced by the fungus in appreciable quantities, but in greater amount when inulin (or a related substance) was present in the culture medium. Within the last year Kopeloff and Byall ('20) studied the invertase activity of the spores of *Aspergillus niger* and *Penicillium expansum* and reported that the maximum invertase activity occurred at concentrations of sucrose between 50 and 60 per cent.

Besides those factors mentioned above, it is also possible that many other chemical "stimuli," H-ion concentration, temperature, etc., may influence the formation of enzymes. From such facts one may infer that certain forms of fungi might, by constant association with one host or certain complicated environmental conditions, change their nature in respect to enzymatic activity and ultimately become quite distinct from other forms of the same species. To what extent, however,

differentiations caused by such environmental conditions might remain as fixed physiological characters of fungi is an open question.

In the experiments which I have performed I had not in mind being able to distinguish species or races in *Rhizoctonia* by such physiological characteristics as variation in enzymic activity. Nevertheless, the use of carefully arranged experiments of this type with synthetic culture media may afford additional opportunities for the identification of strains.

METHOD OF PREPARING ENZYME

The mycelium or felt obtained from a liquid culture was washed several times with distilled water. It was then dried, either by electric fan or heat, and finally transferred to a desiccator. After two days it was accurately weighed and a certain amount of the mycelium crushed and added to a definite volume of distilled water. After being incubated for several hours at 35–40° C., the solution was filtered and a fixed amount of this filtrate employed with the substratum to be studied. The mixed solution was incubated at about 30° C. As an antiseptic 2 per cent toluol was used.

With regard to the proper stage of growth of the mycelium for investigation, Malfitano, in his study of proteolytic enzymes stated that the enzymes showed their greatest activity when the mycelium had reached its maximum growth. Zeller ('16), in his study of *Lenzites saepiaria*, found the greater activity in the mycelium in the case of all enzymes except oxidase, which was greater in the sporophore. Young ('18) also observed that inulase was present in the fungous mycelium in greater amount in the period of sporulation of the fungus and rapidly disappeared after that time.

In my work a qualitative study of the diastase activity in mycelial and sclerotial stages was made. As might be expected, the greatest activity was synchronized with maximum growth. Therefore, in the present investigation it was decided that all the material should be prepared as described as soon as the cultures showed the first sclerotia formation. In general, when the strains are cultivated in potato decoction and kept at 23–25° C., the sclerotia formation of P1 and H was 1 or 2 days

earlier than that of B1 and P4. As a rule P7 produces no sclerotia, so that its mycelium was taken at the time of the collection of material of B1 and P4. Growth of B3 was so slow and poor that sometimes enough material for the investigation was not obtained.

Carbohydrate Metabolism with Special Reference to Cultural and Enzyme Study

Much of the literature dealing with carbohydrases of fungi is discussed in the work of Zeller ('16) and will not be given here. From what has preceded it is apparent that no single temperature adapted for this work would be the optimum for all strains. Nevertheless, 25° C. is favorable for all. Therefore, in the present investigation, if no special mention concerning this factor is given, it will be understood that all the cultures are kept at 24–26° C. After a certain period of incubation the cultures were filtered and the mat of the mycelium washed several times with distilled water and finally dried in the oven after the method described by Duggar and his associates ('17). Each weight was read twice at different times, and an average number was taken for the result. All experiments were made with cultures of the various strains of the same age grown on the same media. The determination of the H-ion concentration, within the limit shown in the following culture media, does not seem to be a limiting factor for the growth of the various forms of *Rhizoctonia*. Therefore in this investigation H-ion concentration may be considered negligible.

STARCH AND SUCROSE

Cultural experiments.—Of each of the following carbohydrates, glucose, cane sugar, and soluble starch, 2 per cent solutions were prepared, and as solvent the well-known Richards' solution, containing as here modified, NH_4NO_3, 1 gm., KNO_3, .50 gm., KH_2PO_4, .25 gm., $MgSO_4$, .25 gm., peptone, 20 gms. and distilled water, 1000 cc.

Twenty-five cc. quantities of each of the above-mentioned solutions were placed in 125-cc. Erlenmeyer flasks. Duplicates of all of these were inoculated with each strain, and the work was done in a culture room in which all dust was thoroughly

precipitated by steam. The result obtained after an incubation of 3 weeks is shown in the following table:

TABLE IV

DRY WEIGHT OF MYCELIUM OF 6 STRAINS OF RHIZOCTONIA

Medium contains	P_H	Strains of Rhizoctonia (gms.)					
		P1	P4	P7	B1	H	B3
Glucose	7.0	0.140	0.145	0.127	0.010	0.147	0.005
Sucrose	7.0	0.185	0.180	0.145	0.010	0.181	0.005
Starch	7.0	0.165	0.195	0.090	0.010	0.160	0.005
Control*	7.2	Negl.	Negl.	Negl.	Negl.	Negl.	Negl.

* No carbohydrate.

At the beginning the mycelial growth of P4 in the sucrose and starch solutions was rather less in quantity than the glucose solution. Gradually, however, the development became more vigorous, and the final results, as shown above, were better than in the latter. The same was true with P1 and H. This relation held true with P7 in the case of sucrose media, but on the media containing starch this strain showed the poorest growth. Growth of B1 and B3 is very scant on any of the media studied, although B1 can grow fairly well on any of the solid media studied.

The course of development just mentioned makes plausible the supposition that the cane sugar and starch were at first slowly converted by these fungi, but inasmuch as the gradual conversion may after a time keep pace with the requirements (where suitable enzymes are secreted), this may be regarded as a favorable factor. The capacities of these forms to produce the necessary hydrolyzing enzymes became a problem of interest in this work.

Growth on potato decoction and starch solution.—Three hundred grams of potatoes were sliced as thin as possible, cooked in 1000 cc. water for 1 hour at about 100° C., then strained through cloth, and 50 cc. of the decoction used in each Erlenmeyer flask. The flasks were autoclaved for 15 minutes at 15 pounds pressure, after which they were inoculated with the various strains. Duplicate flasks inoculated were kept as controls.

After the periods of time mentioned in table v about 5 cc. of the culture solutions for sugar determinations were removed to the transfer room previously mentioned. All the cultures were then again placed in the incubator until the next determination. In this way contamination was entirely avoided and separate flasks were not required for each determination. After

TABLE V

THE GROWTH OF VARIOUS STRAINS ON POTATO DECOCTION*

Strain	Per. of incub. (days)	Growth	Reducing sugar (mgs.)†	Starch remaining
P1	5	2	5.82	4
P4	5	3	9.71	4
P7	5	2	5.80	4
B1	5	1	5.82	6
P1	6	3	5.82	2
P4	6	3	13.59	2
P7	6	3	3.88	2
B1	6	1	10.68	4
P1	8	.043 gm.	5.82	2
P4	8	.043 gm.	5.82	2
P7	8	.055 gm.	3.20	2
B1	8	.010 gm.	6.32	4
P4	12	2	10.14	1
P7	12	2	3.92	2
B1	12	1	14.77	2
B3	12	1	6.32	3
Scl. lib.	12	1	14.77	1
P4	15	3	16.60	0
P7	15	4	5.82	1
B1	15	1	——	1
B3	15	2	20.87	2
Scl. lib.	15	3	21.15	0
P4	25	.160 gm.	.51	0
P7	25	.170 gm.	.54	1
B1	25	.020 gm.	25.67	1
B3	25	.100 gm.	16.45	0
Scl. lib.	25	.175 gm.	.51	0

* Under the columns "Growth" and "Starch remaining" the numerals represent relative amounts, in each case 1 representing a minimum positive quantity.

† Reducing sugar as glucose in 10 cc. of medium.

8 days the experiment was discontinued and dry weights of the mycelial felt were obtained (see table v). The second part of this table represents a repetition of the work, using also *Sclerotinia libertiana* by comparison.

In the next experiments 2 per cent soluble starch was dissolved in the stock solution and 50 cc. of the solution used in the Erlenmeyer flasks as before. In this case, however, 3 flasks were used with each organism.

TABLE VI

SHOWING THE RESULT OF MYCELIAL GROWTH ON SYNTHESIZED CULTURE MEDIA

Strain	Period of exp. (days)	P_H	Growth	Reducing sugar* (mgs.)	Starch remaining
P1	6	5.8	3	1.5	2
P4	6	6.0	2	5.4	0
P7	6	5.8	3	1.6	2
B1	6	6.4	1	1.0	3
H	6	5.8	3	1.6	2
B3	6	6.8	1	0.7	3
Scl. lib.	6	6.8	1	0.4	3
Control	6	6.8	0	.0	3
P1	9	5.6	4	1.6	1
P4	9	5.7	3	5.3	0
P7	9	5.8	4	1.7	1
B1	9	5.9	2	3.9	2
H	9	5.7	4	1.6	1
B3	9	6.7	1	1.0	1
Scl. lib.	9	6.5	2	1.8	1
Control	9	6.7	0	.0	0

* Reducing sugar as glucose in 10 cc. of medium.

From the tables it is to be inferred that all the strains used possess diastase, but no quantitative determinations of the amount of sugar produced could be made, since constant utilization of the sugar occurs.

It is also demonstrated by these experiments that all the strains studied have a general tendency to increase the active acidity during growth, and the rate of its increase is somewhat proportional to the amount of mycelium.

Enzyme study—For the purpose of estimating the enzymatic activity of these strains, the following experiments were carried out by using mycelial extractions added to the substrata to be studied. For sugar estimation the method described by Shaffer ('14) is employed, with slight modification. Two per cent starch solution was prepared, and to 20 cc. of this solution there was

TABLE VII

A QUANTITATIVE STUDY OF DIASTATIC ACTIVITY IN MYCELIAL AND SCLEROTIAL STAGES

Stage of growth	Reducing sugar as glucose in 10 cc. substrate (mgs.)*				
	P1	P4	P7	H	B3
Mycelium	2.54	7.44	2.14	5.95	2.23
Sclerotia	1.98	6.89	2.15	4.84	2.27

* Unless otherwise stated, the amounts of reducing sugar are thus reckoned in all subsequent tables.

TABLE VIII

DIASTATIC ACTIVITY OF THE DIFFERENT STRAINS

Strain	Reducing sugar (mgs.)		Diastatic activity (rel.)	
	After 21 hrs.	After 48 hrs.	After 21 hrs.	After 48 hrs.
P1	7.84	....	30.45	....
P4	25.75	58.41	100.00	100.00
P7	5.88	9.80	22.83	16.78
B1	24.69	41.43	95.88	70.93
H	5.88	9.80	22.83	16.78
B3	11.86	14.90	46.06	25.51
Control*	Negl.	Negl.	0	0
P1	12.35	18.95	63.50	64.38
P4	19.45	29.44	100.00	100.00
P7	10.78	17.90	55.42	60.80
B1	13.84	18.95	71.11	64.37
Control	Negl.	Negl.		

* Starch solution.

added the same amount of .5 per cent mycelial extraction obtained from mats of the fungi grown on potato decoction. Then the mixture was incubated at 29–30° C. for 4 hours when the sugar determinations were made.

The second part of table VIII, below the horizontal line, represents a repetition of most of the work in the first part of the table. From the experiments it is inferred that the diastatic activity of the strain P4 is notable and beyond that of the remaining strains. B1 is next in order of efficiency. The results obtained with P1 and H are rather similar, and follow B1. Diastatic efficiency of P7 is the least of all. This result is confirmatory of the cultural experiments. No definite conclusion may be drawn for B3 owing to the lack of data, but so far as the present experiment indicates the activity of the strain is rather striking and stands above that of P1, H, and P7.

Furthermore, this study was repeated several times with the use of extractions obtained from different cultures or cultures of different ages. In all cases, with the exception of a minor modification, those relationships mentioned above have proved rather constant, and no marked variation was noticed in any of the results. These results were also verified by using the alcohol precipitate of mycelium extractions.

A comparative study of the enzyme from the original as contrasted with the reisolated strains was also made. P1 and P4 were isolated from sclerotia on potato tubers obtained from experimentally infected plants (see inoculation experiment No. 5). The original strain P1 was used for comparison. The result obtained after 5 hours is shown below:

TABLE IX

DIASTATIC ACTIVITY OF ORIGINAL AND REISOLATED STRAINS

	P1	Reisol. P1	Reisol. P4
Reducing sugar (mgs.)	4.78	14.13	15.38

It appears that the diastatic activity of the reisolated strains of P1 and P4 is 3 times as great as that of the original P1. From

this experiment it will be safe to assume that the diastatic efficiency manifested by original strains may be regarded as a permanent and fixed characteristic, but the enzymatic activity may be modified by a transfer to the host plants, while after continual culture in the laboratory it was rather constant on uniform culture media.

With respect to the presence of invertase in these fungi, the following experiments were performed in the same way as decribed above but using 2 per cent cane sugar instead of starch.

TABLE X

INVERTASE ACTIVITY OF THE STRAINS AFTER AN INCUBATION PERIOD OF 21 HOURS AND 2 DAYS

	P1	P4	P7	B1	H	B3	Control	Per. (hrs.)
Reducing sugar (mgs.)	5.39	18.45	14.40	12.80	4.91	5.88	Negl.	21
Invertase activity	29.91	100.00	78.05	69.38	26.61	31.87	0	21
Reducing sugar (mgs.)	7.35	85.38	38.45	91.90	7.35	7.35	Negl.	48
Invertase activity	8.00	92.90	41.84	100.00	8.00	8.00	0	48

This study was repeated several times, using several mycelial extracts obtained from various culture media. With the exception of a slight modification, all the experiments show the same tendency as appears above. All strains possess the power of producing hexoses from cane sugar in excess of the food requirements. Invertase activity of P4, P7, and B1 is relatively high, while that of P1, H, and B3 is poor.

A comparative study was also made of the invertase activity of the reisolated strains of P1 and B1, with the result that these gave respectively 4.38 and 6.11 mgs. reducing sugar as compared with 4.12 mgs. in the original P1.

Maltose and Lactose.—To the stock culture solution previously described 2 per cent maltose and lactose were added, 40 cc. of Sach solution then placed in Erlenmeyer flasks and inoculated. Table XI gives the results after 3 weeks.

As shown by the table, the nutritive value of maltose is about the same as that of dextrose, while that of lactose seems to be much less than that of the other sugars.

For quantitative estimation of maltose activity a 1 per cent solution of maltose was used as a substrate. Twenty cc. of this solution were placed in each of 13 test-tubes, 2 tubes for each organism and 1 for the control. Five cc. of the extractions (0.5 per cent) of the mycelium of the 6 strains were added to each tube except the control which received 5 cc. of distilled water. All the tubes were placed in an incubator (Ca. 28° C.).

TABLE XI

GROWTH OF THE 6 STRAINS ON MALTOSE AND LACTOSE SOLUTION. THE QUANTITIES REPRESENT DRY WEIGHT OF MYCELIUM IN GRAMS

	P_H	P1	P4	P7	B1	H	B3
Maltose	6.8	0.035	0.285	0.237	Negl.	0.330	Negl.
Lactose	?	0.140	0.035	0.065	Negl.	.....	Negl.
Dextrose	7.0	0.332	0.025	0.210	Negl.	0.307	Negl.

TABLE XII

MALTASE ACTIVITY OF THE STRAINS

Strains	Reducing sugar as glucose in 10 cc. substrate (mgs.)	
	After 6 days	After 12 days
P1	52.68	71.45
P4	55.44	72.48
P7	49.58	70.10
B1	58.48	79.22
H	52.95	72.15
B3	52.68	72.15
Control	47.12	48.11

No doubt exists concerning the presence of maltase in all these forms of the fungus, but as shown by the table, no marked specialization is noticed in any of the strains. In this connection it is rather interesting to note that the maltose secretion by the fungi in question bears a close relation to the nutrient solution in which the fungi are grown. In all the strains maltase is produced in greater amounts when maltose is used in the culture media. It seems to me, however, that there is no "qualitative" relation of maltase secretion in these fungi, since under

any condition studied maltase is formed at least in small quantities.

In dealing with lactase the same procedure was carried out as for maltase, except that lactose was used as a substrate. Three experiments with the use of mycelial extractions obtained from mats of the fungi grown on potato decoction were made at different periods, but there is no distinct indication of the presence of the enzyme in an extract from any strain. Nevertheless, the secretion of a lactase by these fungi is suspected because of the constant utilization of lactose when the strains are cultured on that medium. In the hope of securing more definite results in this direction further experiments were made with the 6 strains as shown in table XIII.

TABLE XIII

GROWTH OF THE 6 STRAINS ON MALTOSE AND LACTOSE MEDIA AFTER 4 WEEKS, DRY WEIGHT IN GRAMS

Media	P1	P4	P7	B1	H	B3
Maltose	0.870	0.850	0.670	0.870	0.790	Negl.
Lactose	0.400	0.280	0.640	0.710	0.317	Negl.

From this experiment it appeared that all the strains were able to utilize lactose as a source of carbon, although its availability was much less than that of maltose.

In dealing with lactose the same procedure as described above for maltase was carried out, except that the extracts were added to 2 per cent lactose solutions and incubated for 1 month. Four determinations were made. The results were rather variable, the quantity of K_2MNO_4 required for 2 cc. of the solution varying from 3.2 to 4.1 cc., the experiments indicating that all the strains may contain a small amount of lactase but of low activity.

Monosaccharides.—For these tests 2 per cent glucose, fructose, and galactose dissolved in the stock solution were used, with the conditions as in the previous experiments.

From table XIV it is inferred that all the monosaccharides are directly utilized by the fungi, with approximately equal

availability. No alcoholic fermentation was noticed in any of the strains studied.

Inulin.—With inulin as a source of carbohydrate the 6 strains afforded very small yields ranging from .004 gm. dry weight in the lowest to .015 in the highest. Control cultures with maltose as the carbohydrate nutrient were entirely comparable with the results in table XIV; moreover, no inulase could be demonstrated.

TABLE XIV

THE GROWTH OF SEVERAL STRAINS ON HEXOSE-CONTAINING MEDIA. THE QUANTITIES REPRESENT DRY WEIGHTS IN GRAMS

Media	P1	P4	P7	B1
Glucose	0.425	0.360	0.370	Negl.
Fructose	0.445	0.220	0.352	Negl.
Galactose	0.315	0.247	0.300	Negl.

TABLE XV

THE GROWTH OF THE STRAINS ON AMYGDALIN AND MALTOSE SOLUTIONS. THE QUANTITIES REPRESENT DRY WEIGHT IN GRAMS

Media	P1	P4	P7	B1	H	B3
Amygdalin	.050	.055	.060	.015	.060	....
Maltose	.200	.235	....	0.110	.200	Poor

Amygdalin.—Emulsin was for the first time discovered in fungi in 1893 by Bourquelot, who found it in *Aspergillus niger*, and by Gerad, who found it in *Penicillium glaucum.* The former ('94) was also able to detect emulsin in many higher fungi found on wood. From a variety of experiments performed by many investigators, it has been made clear that many glucosides, such as amygdalin, arbutin, populin, and salicin are attacked by emulsin secreted by certain fungi yielding, of course, glucose as one of the products of decomposition.

In the present experiments 2 per cent amygdalin was added to the stock solution, likewise 2 per cent maltose was used for comparison, 25-cc. quantities being used in the flasks. The results are for a period of 2 weeks, and in general these indicate a low rate of glucoside consumption.

For the emulsin test 2 per cent amygdalin was used as a substrate. Five cc. of this was placed in a test-tube and 2 cc. of enzyme extraction (1 per cent strength) was added to 1 tube of the amygdalin solution serving as control. After incubating for 1 day at 45° C. the reducing sugars were determined by the Fehling test.

TABLE XVI

QUANTITATIVE DETERMINATION OF THE EMULSIN ACTIVITY OF THE 6 STRAINS. THE QUANTITIES REPRESENT MILLIGRAMS REDUCING SUGAR IN 2 CC. SOLUTION

P1	P4	P7	B1	H	B3	Control
5.4	5.6	5.6	5.2	5.5	....	Negligible

In the 5 cases showing positive action the odor of benzaldehyde was easily recognized.

From these experiments it appears that amygdalin is hydrolyzed and then utilized as a source of carbon. It is apparent that while amygdalin was undoubtedly slowly hydrolyzed, its nutritive value is considerably less than that of maltose. Nor was there any marked difference in emulsin activity in the various strains. In connection with this experiment there was obtained for the first time a relatively fair growth of B1 on a maltose medium, while this organism gave no growth heretofore on any of the synthesized culture media. This suggests that the cultural characters of the fungus are by no means invariable, but more or less modified by environment. Subsequent to this experiment the strain B1 was so changed in physiological capacities as to become a very easily culturable form.

Cellulose.—Much of the literature dealing with the solution of cellulose by microörganisms was largely discussed in the

work of McBeth and Scales ('13), Zeller ('16), and Schmitz ('19), therefore no literature review of this subject will be given here.

Filter-paper cellulose was prepared in the manner described by McBeth and Scales ('13). Cellulose agar was prepared by adding to the stock solution about 1 per cent of the precipitated cellulose and 2 per cent agar, afterwards sterilizing as usual. Test-tubes were then prepared with about 15 cc. of the solution and after autoclaving all the tubes were cooled without being slanted, then inoculated.

All the fungi grew relatively well on this medium, dissolving large quantities of cellulose. The hydrolysis of cellulose was shown by the fact that the agar became transparent over an area extending considerably beyond the hyphae.

TABLE XVII

GROWTH OF THE STRAINS ON SYNTHESIZED CELLULOSE CULTURE MEDIA THE DECIMAL QUANTITIES REPRESENT DRY WEIGHT IN GRAMS

Strains	Cellulose		Maltose		No carbon	
	Mycl.	Scl.*	Mycl.	Scl.	Mycl.	Scl.
P1	0.123	1	0.255	3	0.010	0
P4	0.062	1	0.252	3	0.009	0
P7	0.098	0	0.275	0	0.015	0
B1	0.090	2	0.245	3	0.002	0
H	0.048	1	0.245	3	0.012	0
B3	None	0	None	0	None	0

* The numerals in this and in subsequent tables represent relative amounts, 1 being the lowest positive amount, in this case being the minimum of positive sclerotial formation.

For a determination of any specialization of the forms in respect to cellulose utilization recourse was had to a liquid culture medium. This medium was also prepared by adding about 1 per cent of the precipitated cellulose to a complete mineral nutrient solution, the cellulose being the only source of carbon. For comparison cultures were made with 1 per cent maltose and also with the salt solution lacking all carbohydrate. All cultures were incubated for 1 month.

Direct determination of cellulase was made by employing the method described by Zeller ('16). Five-cc. quantities of mycelial extraction (0.5 per cent strength) were added to 10 cc. of the precipitated cellulose solution (about 1 per cent by weight), and the result was determined after 1 month.

TABLE XVIII

QUANTITATIVE DETERMINATION OF SUGARS RESULTING FROM THE CELLULASE ACTIVITY OF THE DIFFERENT STRAINS

	Reduction of Fehling's solution				
	P1	P4	P7	B1	H
A. Enzyme	3	2	2	2	3
B. Enzyme (autoclaved)	1	1	1	1	1
C. Cellulose alone	0	0	0	0	0

In the light of these results there is no doubt that cellulase is present in the mycelium of the strains studied. Its activity was not measured quantitatively, but the cellulase activity of P1 and H is striking as compared with that of the remaining forms.

The 6 strains were grown in Erlenmeyer culture flasks on a mineral nutrient solution containing sucrose and lactose in amounts ranging from 0.1 to 10 per cent. Using the dry weight of mycelium as a criterion there was, as might be generally expected, a progressive increase in yield in all cases except one, up to 5 per cent, of both the sucrose and lactose series, after a growth period of 1 month. The concentrations from 5 to 10 per cent represented a distinct series growing for a period of 5 weeks, and here too there was in all cases with increasing concentration a progressive increase in growth in the sucrose media, the maximum growth occurring in P1 and B1, 0.020 and 1.100 gms. respectively. In the lactose media growth at the higher concentrations was more or less variable, but the surprising feature of these experiments was the high yields obtained at 5 per cent or more, the maximum being 1.05 gms. in the H strain. In general, the limiting concentrations for growth were not determined.

NITROGEN METABOLISM WITH SPECIAL REFERENCE TO CULTURAL AND ENZYME STUDIES

Attention was next turned to the problem of the requirements of the strains for nitrogen. Research has long shown that different fungi and bacteria behave in the most diverse manner in respect to a source of nitrogen, and it seemed that an experimental study of the various strains of *Rhizoctonia* might throw some light on physiological differentiation.

TABLE XIX

THE GROWTH OF THE STRAINS ON VARIOUS NITROGENOUS CULTURE MEDIA. THE GROWTH QUANTITIES REPRESENT DRY WEIGHT IN GRAMS

Solution containing	P_H	P1	P4	P7	B1	H	B3
Amygdalin	6.8	.012	.015	.027	.007	.012	.005
Sclerotia		1	1	0	0	1	1
Asparagin	6.0	.135	.030	.070	.010	.165	.010
Sclerotia		3	3	0	0	3	1
Caffeine	6.8	0	0	0	0	0	0
Sclerotia		0	0	0	0	0	0
Casein	6.0	.155	.120	.090	.520	.165	.110
Sclerotia		3	2	0	1	3	2
Legumin	6.2	.110	.055	.100	.025	.125	.020
Sclerotia		1	2	1	1	1	1
Peptone	6.8	....	.120	.120	.020	.160	.010
Sclerotia		2	2	0	1	2	1

Cultural experiment.—For this experiment .5 per cent solutions of amygdalin, asparagin, caffeine, casein, legumin, and bacto-peptone were prepared, each in a medium containing 2 per cent maltose, .025 per cent magnesium sulphate, and .025 per cent monobasic potassium phosphate. Flasks were arranged with 25-cc. quantities and after inoculation were incubated for 16 days.

Concerning the requirements of nitrogen for each strain, there is some specialization exhibited. As a rule the growth of P1 and H in all the media is more abundant than that of the remaining strains, with the exception of B1 on the casein medium.

With respect to the nutritive value of the media used, P1 and H grew best on casein, peptone, and asparagin, and less on legumin, while P7 grew best on peptone, legumin, casein, and asparagin in the order named. P4 grew best on peptone and casein, showing no apparent difference between the two, and much less on legumin and asparagin. This series of experiments was performed during the earlier part of the investigation. Therefore the strain B1 had not then shown a high capacity for growth on liquid media. The result with this strain on casein was, however, peculiar. B3 was also unable to grow well on any of the media with the exception of casein. As a whole, casein was the most desirable food material for the various strains. Amygdalin, on the other hand, was unsatisfactory as a source of nitrogen, thus bearing out, as far as this experiment may, Nägeli's supposition that nitrogen cannot be assimilated when in direct combination with carbon. Nevertheless, Pfeffer ('99) found that certain fungi were able to grow when supplied with nitrites and might even obtain their nitrogen from amygdalin or potassium cyanide. Caffeine was not utilized by any of the strains.

With respect to the nutritive value of nitrite and nitrate, Pfeffer also states that nitrites are assimilated by nitrite bacteria only, but that they do not serve as a source of nitrogen for mould fungi; yet Winogradsky's observations are not in accord with this view. Many investigations have been made on the comparative value of nitrate and ammonia compounds. It has also been observed that the nitrogen requirements of certain fungi may depend largely upon the source of carbon. For instance, as shown by Fischer, *Bacillus coli* may utilize nitrate in the presence of glucose, but if glycerin is substituted for glucose it does not thrive on nitrate. In the case of *Aspergillus* Czapek showed that the amino acids were preferable to peptone in the presence of glucose.

Although I have not been able to pursue extensively a study of this interesting subject as it relates to *Rhizoctonia*, the following data are interesting as far as they go. In a solution containing 2 per cent maltose, .025 per cent magnesium sulphate, and .025 per cent potassium monophosphate, there were dissolved in different flasks potassium nitrate, potassium nitrite, and potassium cyanide to make concentrations of 5/100 M.

To compare the result with that of organic compounds, 0.5 per cent casein was used in one flask, likewise one flask without a nitrogen-containing compound served as control. Each culture flask, Erlenmeyers of 125 cc. capacity, contained 25 cc. of the medium to be tested, and the incubator interval was 25 days.

TABLE XX

THE GROWTH OF THE STRAINS ON NITROGEN-CONTAINING CULTURE MEDIA. THE DECIMAL QUANTITIES REPRESENT DRY WEIGHT IN GRAMS

Strain	Potassium nitrite		Potassium nitrate		Potassium cyanide		Casein		No. N	
	Mycl.	Scl.	Mycl.	Scl.	Mycl.	Scl.	Mycl.	Scl.	Mycl.	Scl.
P1	.040	0	.210	2	0	0	.310	3	Negl.	0
P4	0	0	.050	1	0	0	.240	1	Negl.	0
P7	0	0	.170	0	0	0	.180	0	Negl.	0
B1	0	0	.020	0	0	0	.040	1	Negl.	0
H	.040	0	.200	2	0	0	.330	3	Negl.	0
B3	0	0	.020	1	0	0	.220	2	Negl.	0

It is interesting that P1 and H are able to utilize nitrogen in the form of potassium nitrite to a certain extent, while the remaining strains can not. The filtrate of the potassium nitrate solution in which P1 and H had grown exhibited a positive color test for nitrite. To what extent, however, this reduction of nitrate and utilization of nitrite may occur with these fungi has not been established. Slight reduction of nitrate was also observed in the potassium nitrate cultures infected with P4 and B1, but absolutely no reduction took place in P7. A solution of 5/100 M of potassium cyanide was rather toxic to all the fungi studied. In general, nitrogen in the form of casein would seem to be most available.

The next experiment was made like the preceding, but with the following inorganic compounds: (1) 5/100 M ammonium nitrate, (2) 5/100 M potassium nitrate, and (3) 5/100 M ammonium nitrate plus 5/100 M potassium nitrate. These compounds were used with 2 per cent glucose in a mineral solution as before.

The highest yield is obtained from potassium nitrate, and the combination of potassium nitrate and ammonium nitrate shows no better result in mycelial and sclerotial growth than the culture which contains either one of these salts. In this experiment P7 shows the highest growth of any of the cultures used.

TABLE XXI

GROWTH OF THE STRAINS ON NITRATES. CULTURE INTERVAL 3 WEEKS THE QUANTITIES REPRESENT DRY WEIGHTS IN GRAMS

	P1	P4	P7	B1	H	B3
NH_4NO_3	.140	.073	.160	.008	.130	Negl.
KNO_3	.183	....	.207	.010	.195	Negl.
$NH_4NO_3+KNO_3$	.140	Lost	.136	....	.140	Negl.

TABLE XXII

GROWTH OF THE STRAINS IN SUGAR BEET DECOCTION AND INORGANIC SOURCES OF NITROGEN. THE DECIMAL QUANTITIES REPRESENT DRY WEIGHT IN GRAMS

Strain	Sugar beet decoction plus 1/100 M.						Sugar beet decoction only	
	Ammonium sulphate		Ammonium nitrate		Potassium nitrate			
	Mycl.	Scl.	Mycl.	Scl.	Mycl.	Scl.	Mycl.	Scl.
P1	.242	2	.172	2	.204	3	.246	2
P4	.062	1	.084	1	.127	1	.237	3
P7	.185	0	.185	0	.252	0	.116	0
B1	.225	2	.233	2	.223	3	.242	2
H	.248	2	.198	2	.226	3	.242	2
B3	.145	1	.028	0	.060	0	.060	0

After conducting a series of experiments in which $(NH_4)_2SO_4$, NH_4NO_3, and KNO_3 in concentrations of M/100 and M/500 were added to sugar beet decoction and the strains grown for 10 days without any apparent advantage from the addition of

these salts, a second series was arranged with a culture interval of 4 weeks. The culture vessels and the quantities of solution were as in the preceding series.

The decoction alone seems not only to afford sufficient nitrogen, but with the exception of P7 the addition of these compounds results in decreased growth.

Proteases.—Experiments were conducted in the usual way using various substrates. As a source of the enzymes mycelial extracts were used in some cases, while in the others the growing fungus was employed.

Action on gelatin.—A 10 per cent solution of gelatin was made up in potato decoction, and about 10-cc. quantities of the solution were placed in test-tubes. The tubes were then immediately autoclaved and slanted. After hardening of the gelatin inoculation was made.

TABLE XXIII

ACTION OF EXTRACTS OF THE STRAINS ON FIBRIN

	Fresh	Fresh	Fresh	Boiled
Substance added Fungus, all strains	Water +	n/10 HCl —	$.5Na_2Co_3$ +	$.5Na_2CO_3$ —

All the fungi grew fairly well on this medium, showing no apparent difference in each strain, and after two weeks all the cultures showed liquefaction of the gelatin. Positive qualitative results were also obtained by using mycelial extracts of all the strains.

Action on fibrin.—The action on this substrate was determined by placing in each tube 5 cc. of the fresh or boiled extract, a piece of fibrin, also 5 cc. of water, acid, or alkali, and then incubating. The behavior of all strains was positive except in the presence of alkali or where the boiled enzyme was used, so that the result may be expressed in a tabulated summary, as in table XXIII.

Action on albumen.—Two per cent of egg albumen was added to a modified Richards' mineral nutrient solution containing

also a 2 per cent dextrose, and this was heated for half an hour in an autoclave. Erlenmeyers of 25 cc. capacity were then inoculated with the 6 strains and incubated at 23° C. for 3 weeks, with the following yields of mycelium: P1, 0.54 gm.; P4, .18 gm.; P7, .015 gm.; B1, .80 gm.; H, .056 gm., and B3, a negligible amount.

A marked hydrolysis of the albumen occurred in P1 and H, with the larger growth quantities.

Growth of the Six Strains in Relation to H-Ion Concentration

The importance of the reaction of the culture media as a physiological factor with microörganisms is well recognized, consequently the literature dealing with this problem is rather extensive. Adequate reviews of the literature dealing with the subject were recently given by Webb ('19), and it is unnecessary to include such reviews in this paper. Concerning the influence of acid and alkali on the growth of these fungi, however, Duggar ('99), in an early paper referring to remedial measures, made the following statement: "The use of an alkali as a preventive might be logically suggested knowing the rapidity with which the fungus grows on acidulated nutrient media." Peltier ('19), however, in his study on carnation stem rot stated: "The results showed that *Rhizoctonia* can grow on medium which is, within reasonable limits, either acid or alkaline in reaction." No definite determination of the effect of hydrogen ion (or hydroxyl ion) concentration upon the rate of the mycelial growth of these fungi has been made.

In my investigation, although rather preliminary in nature, the attempt has been made to determine the critical and optimum H-ion concentration, such as observed by Meacham ('18) in mycelial growth of four wood-destroying fungi, or by Webb ('19) in the germination of the spores of certain fungi; likewise I have endeavored to ascertain whether there is any specialization of strains in this respect.

In my earlier studies during this investigation the reactions of the various natural decoctions were designated by Fuller's scale. That this is unsatisfactory is now well known (Duggar and associates, '17), so that in the later studies the method of hydro-

gen-ion concentration (Clark and Lubs, '17) was employed, in the manner detailed below.

Experiment 1.—Potato decoction was prepared as usual, then neutralized by means of NaOH. The desired reaction was obtained by the addition of HCl or NaOH, the procedure being then, as in many previous cases, to employ 25 cc. in flask cultures. After autoclaving, the actual H-ion concentration was determined with the use of a colorimeter as outlined by Duggar and Dodge ('19), subsequently by Duggar ('19).

The results after 3 weeks' incubation at 25–28° C. are given in the following table. The growth quantities presented in the table are invariably an average of 2 cultures.

TABLE XXIV

THE EFFECT OF H-ION CONCENTRATION ON THE GROWTH OF THE STRAINS IN POTATO DECOCTION, THE UPPER SERIES AT ROOM TEMPERATURE AND THE LOWER AT 30° C., DRY WEIGHT IN GRAMS

Strains	P_H 2.6	P_H 3.9	P_H 6.0	P_H 7.0	P_H 8.2	P_H 8.9
P1	No	0.128	0.076	0.080	0.074	0.040
P4	No	0.095	0.084	0.078	0.060	0.035
P7	No	0.070	0.070	0.060	0.050	0.030
B1	0.080	0.146	0.084	0.092	0.060	0.020
H	No	0.110	0.083	0.080	0.069	0.040
B3	No	Negl.	Negl.	Negl.	Negl.	Negl.

Strains	P_H 2.8	P_H 3.8	P_H 4.3	P_H 5.7	P_H 5.9	P_H 6.7
P1	0.100	0.088	0.080	0.070	0.060	0.055
P4	No	0.080	0.054	0.065	0.060	0.051
P7	No	0.085	0.060	0.065	0.060	0.055
B1	0.125	0.095	0.075	0.060	0.068	0.060
H	0.100	0.090	0.080	0.060	0.070	0.060
B3	No gr.	No gr.	No gr.	No gr.	No gr.	No gr.

Rather extensive observations were made on the series kept at 30° C., as follows: By the second day, the growth of P1, B1, and H was evident in the cultures of P_H3.8, 4.3, and 5.7, while after 5 days the same strains showed growth at P_H5.9 (natural solution) and P_H6.7.

Experiment 2.—Sugar beet decoction was used in the same manner, and the result after a growth period of 3 weeks is shown in table XXV.

All the strains, in both experiments, showed an increased growth on the acid side. In the first experiment maximum growth of all the strains was obtained where the exponent was P_H 3.9, the highest acidity in the cultures used was P_H 2.6, and no growth was obtained at P_H 2.6 except with B; while, on the other hand, as shown in the second experiment, no notable differences in growth occurred between the exponent 3.0 and 7.0, although at P_H 4.4 there is a slight increase in all of the strains.

TABLE XXV

THE EFFECT OF H-ION CONCENTRATION ON THE GROWTH OF THE STRAINS IN SUGAR BEET DECOCTION, THE DECIMAL QUANTITIES REPRESENT DRY WEIGHT IN GRAMS

Strains	P_H 3.0		P_H 4.4		P_H 5.5		P_H 7.0		P_H 8.0		P_H 8.6	
	Gr.	Scl.	Gr.	Scl.	Gr.	Scl.	Gr.	Scl.	Gr.	Scl.	Gr.	Scl.
P1	.200	2	.225	3	.225	2	.220	2	.180	2	.155	2
P4	.185	2	.190	4	.195	3	.190	3	.160	3	.140	2
P7	.200	0	.170	0	.145	0	....	..	.105	0	.090	0
B1	.210	2	.225	3	.225	2	.220	2	.200	2	.190	1
H	.220	2	.250	3	.245	2	.230	2	.190	2	.160	2
B3	.185	2	.175	2	.180	2	.180	2	....	..	.060	1

No explanation of the diversity of results in these two cases can be given, but my suggestion is that the effect of the H-ion concentration may be more or less related to the availability of the food materials of the media, since we know that the sugar beet decoction is much more desirable for these fungi than the potato decoction.

Experiment 3.—A modified Richards' solution was prepared which contained 1 gm. ammonium nitrate, 0.5 gm. potassium nitrate, 0.25 gm. potassium monophosphate, 0.25 gm. magnesium sulphate, and 20 gms. dextrose in 1000 cc. of water, and hydrogen-ion concentration was adjusted by adding H_3PO_4 or NaOH. Growing the strains as before the results after a growth

interval of 4 weeks are shown in table XXVI, from which it appears that on this medium there is a narrow range of reaction for favorable growth. Sclerotial formation occurred only at P_H4.4.

Sclerotia formation in B1 occurred at P_H 3.8, also P_H 4.3, and much less in the natural solution; but no sclerotia were formed at P_H 6.7. From P_H 2.8 or 3.8 growth declined more or less consistently in all strains, as the H-ion concentration was diminished. The most notable result among these observations, however, is the extensive growth of B1, after 5 days, apparently due to the higher temperature. The incubator chamber was then lowered to about 24° C., and the third observation was made after an interval of 12 days. Some new growth of B1 was then apparent at P_H 2.8, in which hydrogen-ion concentration the remaining strains had failed to make growth. At this time

TABLE XXVI

THE EFFECT OF H-ION CONCENTRATION ON THE GROWTH OF P1 IN A SYNTHETIC NUTRIENT MEDIUM

Reaction	P_H 2.0	P_H 3.0	P_H 4.4	P_H 6.8	P_H 8.2
Dry wt. (gms.)	No	No	0.055	0.025	0.010

sclerotia formation of B1 was abundant at P_H 4.3, and less at P_H 3.8, and the same was true of P1 and H, although the rate of growth was much lower. The result after an incubation interval of 4 weeks is shown in the table, and further discussion is unnecessary.

The effect of H-ion concentration on the growth of the strains is rather variable, or rather somewhat related to the media on which the fungi grow. It is almost impossible therefore to name a definite optimum; nevertheless, the following tentative conclusions may be drawn: No marked specialization as to favorable H-ion concentration was observed, although B1 had wider range on the acid side; and (2) in general, these fungi grew well on acidulated media, as observed by Duggar ('99), the favorable hydrogen-ion concentration being about P_H 3.8.

Fusion of Hyphae

Fusion of hyphae is common in this fungus and may be observed in any culture of the strains studied, especially in the young stages. It is generally considered that the fusion of hyphae occurs either between hyphae of the same parent mycelium or between hyphae arising from separate colonies of the same physiological form. But with regard to fusion between the different strains obtained from different plants of the same host species, or from different hosts, practically no attempt has been made by any of the earlier investigators to determine this point. I have obtained some interesting results, so that a brief mention of these and of the methods involved is necessary.

For direct study the ordinary drop-culture method was employed, and as a medium 5 per cent maltose in water was used. Hyphae or sclerotial aggregates of the same strain or of different strains were separately sown at opposite sides of a drop. All the cultures were incubated at 27° C., and careful and continual microscopical observation was necessary, because the hyphae or sclerotia may grow out in a few hours, then branch profusely, and readily mingle with the hyphae of the other colony or strain.

In any case, when two hyphae of the same strain are sown in a drop, fusion of hyphae readily takes place. In general, there is a slight inhibition of growth at the margin of the two colonies. Fusion of hyphae occurs readily when P1 and H are sown in the same drop culture. With P1 and P4 the growth rate is about the same, and fusion may take place, although it is not so frequent as in the case of P1 and H. There is no fusion of hyphae between P7 and any of the remaining strains. When P1 and B1 are sown together and incubated at about 21° C., the growth of P1 is much more vigorous than that of B1 and the growth of the former inhibits the further growth of the latter; while if the cultures are incubated at a higher temperature (about 28–30° C.), the result is opposite. In no case does fusion of hyphae occur. A precisely similar result is obtained when B1 and H are grown together. No fusion of hyphae has been established between B1 and any of the remaining strains. When B3 is sown opposite to one of the remaining strains, the growth of the first-named is always inhibited by the latter, so that up to the present time no definite data can be given.

When P1 is sown with the reisolated H (see inoculation no. 1) the growth of both strains is about the same, and frequently fusion of hyphae takes place. Fusion was also frequent between two reisolated strains of P1 and P4, both of which were originally isolated from potato tuber (see inoculation no. 5). In no case was the fusion of hyphae observed when P7 was sown with one of these reisolated strains.

These experiments establish the fact that fusion may occur either between hyphae of the same strain or between those of certain different strains. It is not to be inferred that this fusion phenomenon is analogous to that occurring in *Mucor* or other species of *Zygomycetes*. In the cases here reported the fusion took place only between the hyphae of strains which might possibly have originated rather recently from the same form or race, although modified by environmental conditions. The process by no means represents sexual union. This view is also confirmed by certain experiments with mixed cultures, in which the method described by Zeller and Schmitz ('19) was employed, with slight modification. Petri dishes containing sugar beet agar were each inoculated with 3 of the strains in such a way as to have all possible combinations of each. P1, B1, and H grew much more rapidly than the remaining strains, frequently covering the latter. When hyphae of the same strain, but representing different isolations as P1 and H, came in contact, there was usually no influence of the one colony on the other; that is, the mycelium of the two colonies intermixed, showing a straight line at the margin of the two colonies, owing to the slight inhibition of growth. When B1 and any of the remaining strains came in contact there was a slight stimulation of sclerotial formation on the side of B1 at the margin of the two colonies, shown by the heaping up of sclerotia, while P1 and H (or certain other strains) had rather a tendency to produce sclerotia at the opposite side of the contact line.

The influence of one strain upon another was also studied by another method. After considerable growth of these strains on agar plates the agar-penetrated layer was cut into squares (about 8 mm. across) at the border of any two colonies in such manner that each square contained approximately the same amount of the two colonies. Freshly poured agar plates were

then inoculated with each one of these squares. In cases where the plates were inoculated with the squares of P1 × P4, P1 × P7, H × P4, or H × P7, the growth of P4 and P7 was always inhibited by the accompanying strain and no further growth was noticed, while, on the other hand, when the squares with P1 × B1 or H × B1 were used, the results were entirely dependent upon the environment. For instance, if the plates were immediately incubated at rather high temperatures, growth of the strain P1 or H was inhibited by the strain B1, and the plates were entirely covered by the latter, while if the cultures were incubated at a rather lower temperature, quite the opposite result was noticed. In no case was there evidence that heterozygosis occurred. Growth of the strain B3 was very slow and always covered by growth of the other strains so that no data may be secured for this form in contact with others.

In spite of the data presented, these results must be regarded as preliminary, and in further work cytological technique should be employed.

Aëration

With various fungi experiments have shown that the effect of inadequate aëration is repression of growth or suppression of fruiting stages. In order to determine the relation to aëration in *Rhizoctonia* a series of flasks of different sizes were used. Fifty cc. quantities of sugar beet decoction were pipetted into each flask. Immediately after inoculation with the 6 strains the cotton plug was pushed slightly down into the neck of each of the flasks and then sealed with melted paraffin. The results after one month are as follows:

TABLE XXVII

THE INFLUENCE OF AËRATION ON GROWTH AND SCLEROTIAL FORMATION, DRY WEIGHT IN GRAMS

Strains	Size of flasks									
	1000 cc.		500 cc.		250 cc.		125 cc.		Control, 125 cc.	
	Growth	Scl.	Growth	Scl.	Growth	Scl.	Growth	Scl.	Growth	Scl.
P1	.205	Rare	.090	No	.025	No	.020	No	1.065	
P4	.145	Rare	.040	No	.020	No	.015	No	.540	
P7	.180	No	.085	No	.030	No	.010	No	.820	No
B1	Negl.	No	Negl.	No	Negl.	No	Negl.	No	Negl.	No
H	.225	Rare	.100	No	.025	No	.020	No	.980	
B3	Negl.	No	Negl.	No	Negl.	No	Negl.	No	Negl.	No

The result is so clear as to leave little doubt of the suppression of growth and sclerotial formation owing to the sealing of the flasks. At the same time, however, it should be noted that at the close of the experiment the check flasks were always almost dry, so that the humidity conditions as well as the concentration were different from those of the sealed flasks.

INOCULATION EXPERIMENTS

Experiment 1 (*a*). (*Inoculation of "navy beans" with various strains of Rhizoctonia*).—A number of bean seedlings, each in a pot of sterile soil, were inoculated with the 6 strains by placing some mycelium (all from potato cultures of the same age) near the plants about one-third below the surface of the soil. Twelve plants were inoculated with each strain. After a week "damping-off" was noticed in the pots inoculated with P1 and H, 9 plants being affected in each case. The strain B1 was also able to infect the host to a certain extent, as 3 seedlings were affected. The pots inoculated with P4, P7, and B3 were healthy after 2 weeks.

(*b*). (*Inoculation of navy bean plants with P1, B1, and H*).—Young beans 7 inches high were pricked with a sterilized scalpel and inoculated with each of the 3 strains mentioned, 10 plants being used in each lot. All the plants were supported by bamboo sticks so as to grow erect. Many of the plants were affected, and with such old beans discoloration is observed not only on the infected stems but also on the roots, yet in no case were the plants killed. Pods were also affected, and through the sunken areas of these the hyphae penetrated the seeds and produced small sclerotia on the seed-coats. No distinction between these 3 strains was noticed in symptoms nor in cultural characters.

Experiment 2. (*Inoculation of Lima beans with the six strains*).—Young beans, 5 inches high, wounded by a sterilized scalpel, were inoculated with the 6 strains, 10 plants being used for each strain. After a week distinct reddish brown lesions of various sizes were produced just at the wounds of those plants inoculated with P1, B1, and H, while on the stem inoculated with P4 and P7 only very slight discoloration, with a light-colored sunken area, was noticed. In the check plants only brown punctures resulted, without any further development of lesions. In

general, when the strains attack such old seedlings as were used in this experiment, no pronounced symptoms are observed. In spite of the lesions mentioned most of the plants seemed to be quite healthy. However, such plants have a tendency to break easily in the region of the infected part.

Experiment 3 (*Inoculation of navy beans with the six strains*).—Seeds used for the experiments were soaked in 10 per cent Javelle water for 2 hours and dried at room temperature. The seeds were sown with the mycelium of the 6 strains in sterilized pots. The final observations were made after 3 weeks, and are given below.

TABLE XXVIII

INOCULATION OF NAVY BEANS

	P1	P4	P7	B1	H	B3	Check
Seeds used	20	20	20	20	20	20	20
Seeds germinated	2	9	11	8	1	10	10
Number damping off	2	0	0	1	1	0	0

TABLE XXIX

INOCULATION OF LETTUCE

	P1	P4	P7	B1	H	B3	Check
Number of plants used	12	11	12	12	12	12	12
Number damping-off	9	2	0	4	11	0	0

Experiment 4 (*Inoculation of lettuce with the six strains*).—Young plants, 2 inches high, were inoculated with the 6 strains by placing some mycelium obtained from potato cultures of the same age near the plants about one-third inch below the surface of the ground. For 2 days all the plants were covered with wet newspaper, and the results above are after 10 days.

Experiment 5 (*Inoculation of potato tubers with the six strains*).—The tubers were sterilized with formalin and inoculated with

the various strains. Each tuber was planted in a pot of sterilized soil and placed in the greenhouse. The results are shown in table XXX.

After 4 months all the plants were dug up, and the tubers very carefully examined. Practically all those from the sections P1, P4, and H presented more or less sclerotia on the surfaces showing apparently no difference in color and form. Numerous sclerotia were also observed on the stolons.

TABLE XXX

INOCULATION OF SEED-POTATOES

	P1	P4	P7	B1	H	B3	Check
No. seed-potatoes used	10	10	10	10	10	10	10
No. potatoes sprouted	8	9	9	10	9	10	10
No. stem lesions	1	1	2	0	2	0	0
No. plants, black speck sclerotia	3	3	0	0	2	0	0
No. plants, stem lesion and black speck	4	2	0	0	3	0	0

In pathogenicity as well as symptoms no marked distinction between P1 and H was noted. Concerning the relationship between P1 and P4 as stated in the section dealing with the cultural experiments, these are not considered distinct species, but may be regarded as only somewhat specialized in physiological and morphological characters.

P7 is not virulent, although it may induce some secondary injury when the plants are physiologically weak. No light is thrown on the relationships between P1 and B1, for absolutely no infection occurred in the section B1. In general, none of these fungi kill the host plant directly under the conditions described.

Experiment 6. (*Planting the tubers showing Rhizoctonia sclerotia*).—Twenty tubers showing *Rhizoctonia* sclerotia were planted in pots of sterilized soil. The plants were removed from the pots and examined after about 4 months. Many of the tubers were then covered with sclerotia, but no other symptoms, such as stem lesion, *Rhizoctonia* scab, etc. were noticed. As a matter of fact, the tubers produced in the pots were very

small or somewhat deformed, but probably not caused by the fungus, since those in the check were similar in type.

Experiment 7. (*Inoculation of egg-plants with the six strains*). —Sterilized pots with a few egg-plants (1.5 inches high) were inoculated below the surface of the soil, as in many previous cases, with the 6 strains. After 2 weeks the results observed were as follows.

TABLE XXXI

INOCULATION OF EGG-PLANTS

Fungus	P1	P4	P7	B1	H	B3	Control
No. plants inoculated.....	20	18	16	18	15	10	5
No. plants damping off.....	18	10	None	9	10	None	None
Per cent diseased.....	90	56	0	50	67	0	0

TABLE XXXII

INOCULATION WITH ORIGINAL CULTURE MATERIAL OF P1

Plants inoculated	No. plants used	No. plants infected
Potato (10 days after sprouting).....	10	5 (stem lesion)
Lettuce (about 2 inches).....	20	13
Egg-plant (about 2 inches).....	20	12
Navy beans (about 2 inches).....	15	7
Lima beans (about 2 inches).....	13	6

In general, there were notable differences in the pathogenicity of the different strains, and this was rather consistent for each strain. In every case the virulence of the strains of P1 and H was remarkable as compared with that of the remainder. Nevertheless, I have still some doubt whether the tendencies manifested by the different strains are sufficiently distinctive to be considered as the fixed hereditary characteristics of those strains. Studies in that direction might throw some further light on the differentiation of the strains.

In order to throw further light on specialization in respect to pathogenicity as a factor which might or might not be readily

intensified, the experiments below were arranged. A piece of agar with mycelium was placed in the soil at a distance of about 1 inch from a plant to be inoculated. No wound was made.

TABLE XXXIII

INOCULATION WITH REISOLATED STRAINS OF P1

Plants inoculated	Reis. strain from	No. plants	No. infected	Remarks
Potato (1.5″ high)	Potato	10	7	Stem-lesion
	Lettuce	10	5	Stem-lesion
	Egg-plant	10	4	Stem-lesion
	Navy beans	10	6	Stem-lesion
	Lima beans	10	4	Stem-lesion
Lettuce (about 2″)	Potato	20	19	
	Lettuce	10	19	
	Egg-plant	10	18	
	Navy beans	18	14	
	Lima beans	17	9	
Egg-plant (about 2″)	Potato	20	10	
	Lettuce	20	12	
	Egg-plant	20	15	
	Navy beans	20	13	
	Lima beans	20	9	
Navy beans	Potato	15	6	
	Lettuce	13	6	
	Egg-plant	15	6	
	Navy beans	15	9	
	Lima beans	15	8	

As shown by the table, the pathogenicity of the fungus is more or less modified by changing the host plants on which it lives. The highest pathogenic efficiency is always secured when an inoculation is made on plants belonging to the same species as the host from which the inoculation material originated.

PARASITISM OF RHIZOCTONIA, WITH SPECIAL REFERENCE TO PENETRATION OF HYPHAE

Concerning the nature of parasitism in *Rhizoctonia*, Drayton ('15), in his microscopical examination of transverse and longi-

tudinal sections of the diseased potato stem, showed that cells of the cortex, vascular bundles, and pith were all found to be invaded by mycelium, and finally the vessels became stuffed with the fungus so that one might infer a lessening of the transpiration stream, resulting in undersized tubers or curling of the leaves, etc. Lastly, he states: "Naturally, sometimes infection may be slight and no leaf curling will have occurred, but the evidence offered is sufficient proof for the stem parasitism of the fungus."

Following his work a study of the parasitism of the potato *Rhizoctonia* was also undertaken by Güssow ('17). He states that tips of the young rootlets fall victim to the invading mycelium, the short shoots are destroyed, and the final effect is decreased. Moreover, since the growth of the soil tubers is precluded the production of aërial tubers may occur.

Other papers touching upon this aspect of the subject (Duggar and Stewart, '01; Fulton, '08; and Barrus, '10) are either of minor importance or have been reviewed by Drayton ('15).

From the facts referred to, it will be inferred that the forms here discussed are able to penetrate practically all living plant tissue and to cause disease in the host. Concerning the mechanism of the penetration of the tissue by the hyphae, however, practically no work has been done on this organism. Well known is the work of earlier investigators, especially that of DeBary ('86) on *Sclerotinia libertiana*, who used in part the droplets exuded from the fungus, and concluded that the breaking down of the cell walls was due to an enzyme secreted by the fungus. Ward ('88), studying the *Botrytis* on lily, confirmed DeBary's view, finding that the fungus excreted relatively large quantities of enzyme and dissolved its way into the cell wall. The same opinion was maintained by Büsgen ('93), who maintained that penetration of wall and cuticle by *Botrytis cinerea* is not by mechanical means alone. Nordhausen ('98) also agreed with DeBary, but he found that under certain conditions oxalic acid might play a role in dissolving the cell wall. Smith ('02), following the work of Nordhausen, confirmed the responsibility of oxalic acid in destroying the cell wall.

Brown ('15), studying *Botrytis*, affirmed the enzyme viewpoint, but at the same time he found that the fungus excreted a toxic

substance, which was not of the nature of oxalic acid nor an oxalate, as Smith and others claimed.

Concerning the penetration of epidermal cells by the fungus last mentioned, Blackman and Welsford ('16) showed very clearly that this fungus bores through the cuticle in a purely mechanical way. In his second paper Brown ('16) is convinced that, "the infecting germ tubes of *Botrytis cinerea* are unable to affect chemically the cuticle of the host, nor do they secrete any toxic substance which can pass through the cuticle and bring about the death of the underlying cells." When the cuticular obstacle has been penetrated in a purely mechanical way, the underlying tissues are entered.

Büsgen ('18) published a further study on *Botrytis cinerea*, and from an abstract of this paper it appears that he found no cuticular lesions; the cell walls of invaded cells were more or less dissolved, nucleus and chlorophyl bodies were mostly intact, and the fungus produced a poison not of the nature of an enzyme.

More recently Hawkins and Harvey ('19), in a physiological study of *Pythium*, conclude that the fungus secretes a toxin which kills the cells of the potato, likewise an enzyme by which the organism breaks down the middle lamellae of the host cell, but mechanical pressure exerted by the fungous hyphae seems to be the most important factor in cell wall penetration.

METHODS IN THE STUDY OF HOST PENETRATION

In the studies on penetration, most of the investigators mentioned above made use of sections of different parts of the host plants obtained as nearly sterile as possible and then incubated with the fungus. This method of study, however, did not seem applicable in my investigation, because under such conditions penetration would take place much more easily than under natural conditions, and the degree of the parasitism of the different strains would not be ascertainable. The study was therefore undertaken under conditions as near natural as possible.

The method employed in the present experiment was as follows: Seed disinfection for pure culture work was carried out after the method of Duggar and Davis ('19). About 30 seeds of the garden pea were placed in a small cheese-cloth bag, and these immersed in a covered vessel containing 10 per cent Javelle

water. After a treatment of 4 hours the bag was transferred for a few minutes to a jar containing sterile water and then again rinsed in a second jar. The contents of each bag were carefully emptied into a sterile Petri dish. The seeds were then transferred to Petri dishes containing potato decoction agar. All these treatments were made in a transfer room in which all dust was thoroughly precipitated by steam.

The dishes were then incubated at about 25° C. After germination took place each one of the seeds free from contamination was transferred to a large sterile test-tube (1 inch in diameter, 8 inches in height) containing damp filter-paper at the bottom. The basal part of the tube was then covered with black paper to prevent exposure to sunlight. All the cultures were then placed in a greenhouse, and after a few days, when the plants had grown to 2 inches, inoculation with the different strains was made.

The first appearance of the disease was generally observed after a few days, showing a remarkable discoloration in the part attacked. The first sign of the disease in the plants inoculated with P1 and H usually appeared at least 1 or 2 days earlier than that in the plants inoculated with P4 or B1. In any case, no disease was produced by B3. The strain P7 is of low virulence, and if it may attack the plants the first symptom appears later, usually after 2 or 3 weeks. In general, the fungi attacked the plants both on stems and roots, but a direct attack of leaves was rather infrequent.

From the microscopical investigations it appears that the hyphae may enter the plant directly through the surface and in no case through natural openings (fig. 5a and b). Of course, it should be remembered that the stomatal condition is greatly modified by environment, consequently the evidence obtained from the saturated conditions of the cultures cannot be readily applied to natural conditions.

Attention would then naturally turn to the question whether the penetration of the epidermal cell wall is effected in a purely mechanical way or by some special secretion of the fungous hyphae. To solve this question a few drops of mycelial extraction of P1 were put on the upper surface of a pea leaf which was placed in a sterilized Petri dish. For comparison another leaf

was infected with the mycelium of P1 obtained from a pure culture. All the leaves used in the experiment were previously washed in running water for an hour, and finally rinsed with sterilized distilled water in a culture room. All the dishes were then placed in an incubator.

After 2 days some discoloration appeared in the leaves inoculated with mycelium, but practically no change was noticed in the leaves treated with mycelium extract. Microscopical investigation also clearly demonstrated that the tissues of the leaves infected with the mycelium was more or less invaded by the fungus and disorganized to a certain extent, while in the other practically no change had taken place. So far as present information goes, this fact may be cited in support of the mechanical theory proposed by Blackman and Brown.

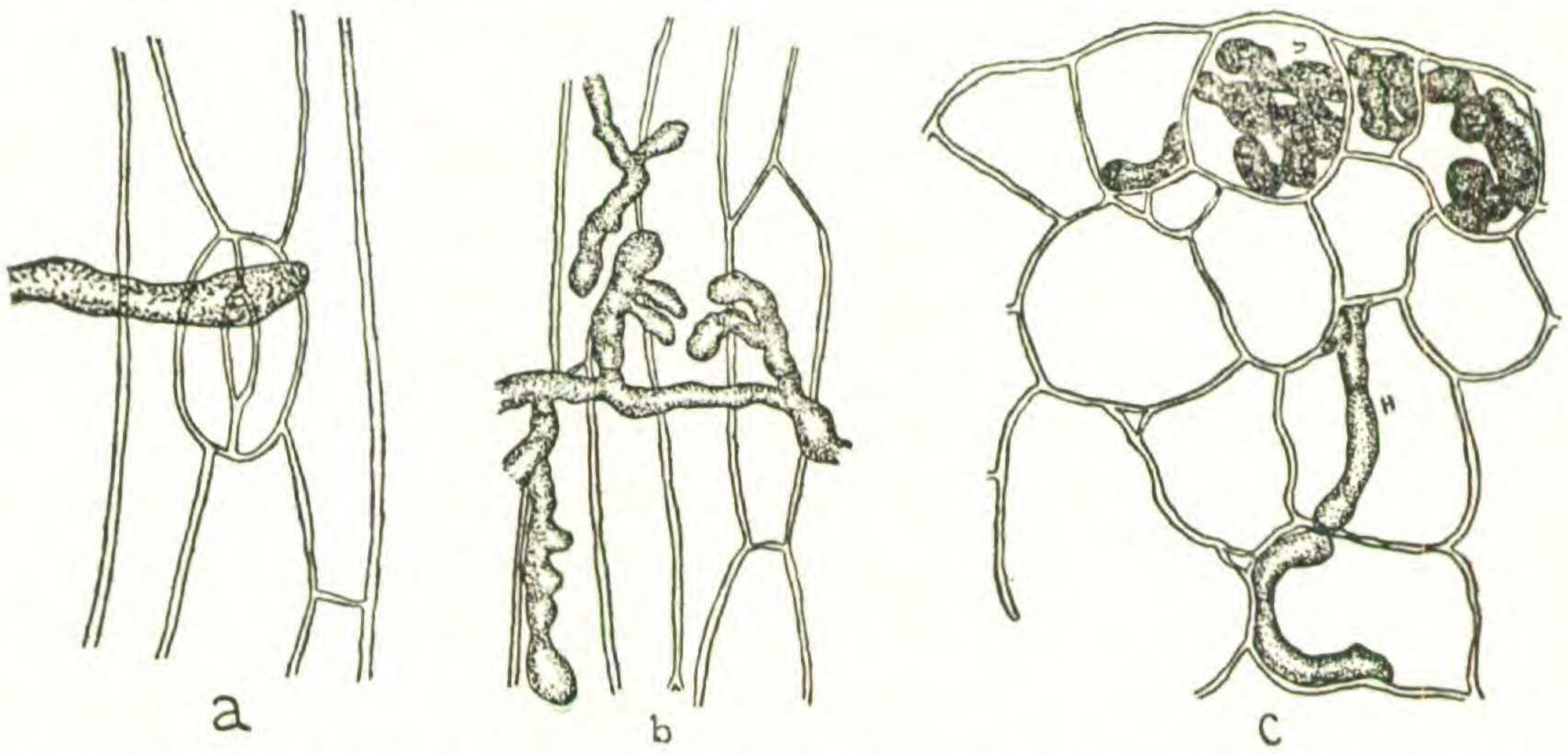

Fig. 5. P 1 *a*, hypha not entering through stomata; *b*, swelling of hypha in contact with epidermal cells of pea stem; *c*, penetration of cell wall of pea root by invading hypha (H), young stage of sclerotia formation (S). (Camera lucida drawings.)

If the fungus in question attacks the plant in a purely mechanical way, as discussed above, it would be logical to believe that the infection of the plant by the fungus might take place much easier at the roots than anywhere else, since the epidermis of the roots, unlike that of leaves and stems, has no cutinized walls, especially in the young stage. My attention was, therefore, turned to this point.

For the purpose of this study the following was devised: Young pea plants (more than 4 inches high) grown in pure cultures in test-tubes were pulled out by means of forceps in such a

way as to leave only the roots in the tubes. Then some of these plants were inoculated by placing the mycelium of P1 on the surface of the young roots, after which each plant was supported in the culture tube by means of a sterilized cork at the base of the stem, and the cork was inserted in the tube. To close any air connections between the inside and outside of the tube, the cork was sealed with melted paraffin. The remaining plants were also treated as above, except that the plants were inoculated on the stems instead of on the roots. All these operations were performed in a culture room. The cultures were then removed to a greenhouse and placed under a glass hood, and the atmosphere maintained at the saturation point during the infection period.

Owing to the lack of water in the tubes, the observations were only continued 2 weeks. A few days after inoculation striking brownish discolorations were noticed on the roots inoculated with the fungus, while the plants inoculated on the stems exhibited no positive symptoms. In general, stem infection may take place only in cases where the plants are very young (about 1 or 2 inches high), while the roots are immediately attacked by the fungus. Especially do the young rootlets soon fall victim to the invading mycelium, as observed by Güssow in his study on potato *Rhizoctonia*. Finally, of course, the aërial parts of the plants wilt, because of lack of water supply through the affected roots. As a matter of fact, the loss of water from the tubes containing plants infected through the roots is extremely slight as compared with the others.

Interest was next centered upon the mechanism of penetration of the hyphae after passing through the cuticle. It might be assumed that the penetration of the cell wall is effected by enzymatic action, since these fungi are able to secrete an enzyme which breaks down pure cellulose. Nevertheless, there was still the necessity of determining this point positively. Many sections of roots infected by P1 were made by the method described by Vaughan ('14). Evidence was obtained indicating that the hypha forms a swelling at the end, as it comes in contact with the cell wall, and that it penetrates the wall by a small tube, after which the penetrating hypha usually reassumes its normal diameter. The most interesting feature was that the

hypha usually obtains entrance at a corner where two cells are in contact, in which case the invading hypha frequently grows between the cells dissolving the middle lamella. As shown by some figures (fig. 5c, fig. 6a–d) made at the time of observation, there are also many cases which clearly indicate distortion of host cells following the penetration of the hypha.

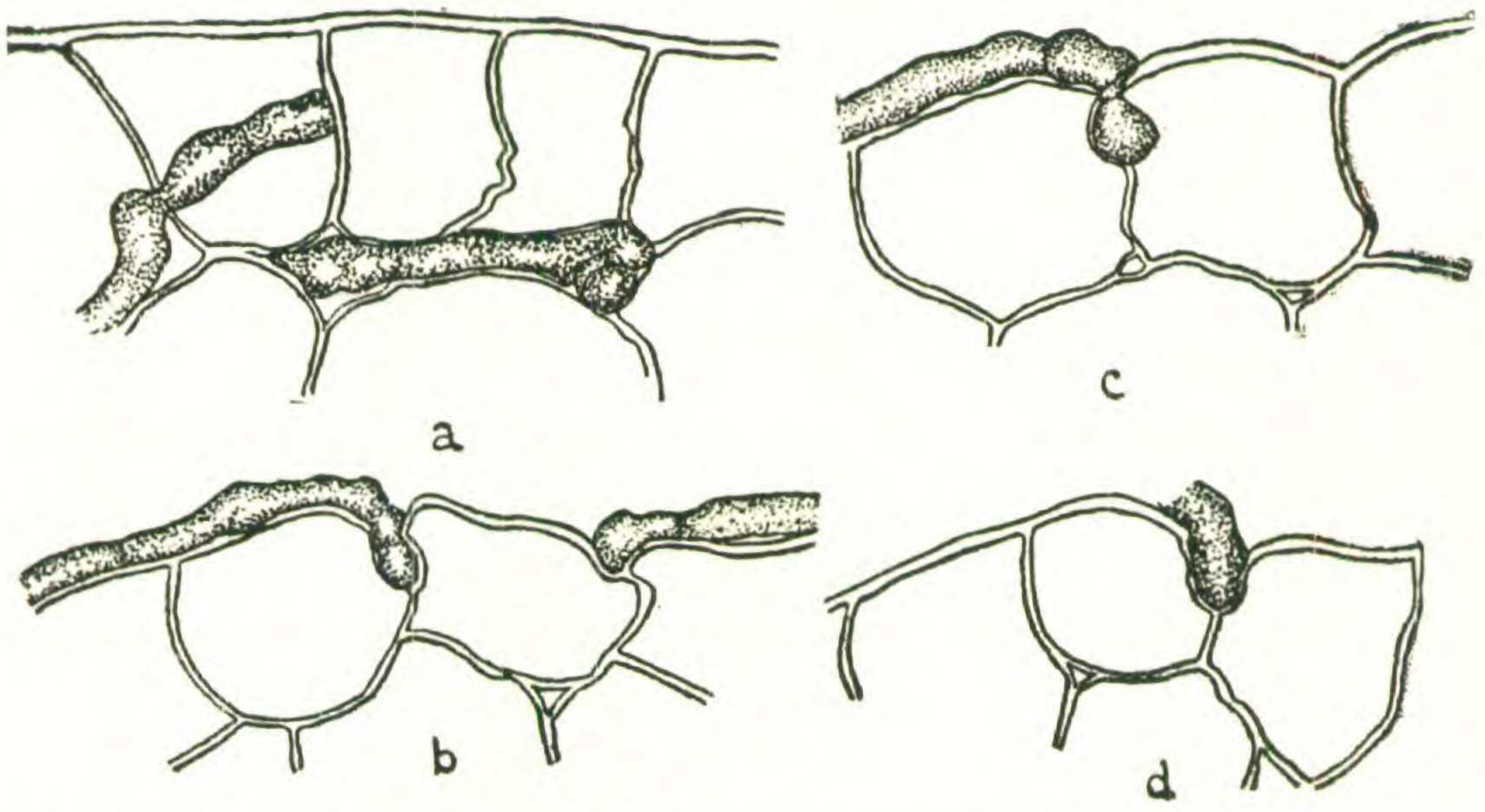

Fig. 6. Camera lucida drawings showing penetration of epidermal cells of pea roots.

From these observations I am of the opinion that the penetration of cell walls may not take place merely in a chemical way, but rather assisted by the mechanical pressure exerted by the fungus. Penetration of the hyphae of P4 and B1 was also noticed to a certain extent, while P7 and B3 seemed to be quite unable to penetrate any living tissue.

DISCUSSION OF DATA

From all the evidence at hand it appears that P1 and H are quite identical in their morphological and physiological characteristics, as well as in pathogenicity, and should properly be included under one form of the species *Rhizoctonia Solani* Kühn. This form is a very common type, and the cause of very serious diseases of many cultivated plants. It is very interesting to notice that these two strains, here shown to be identical, are of different geographical origin and from different host plants. I am also inclined to believe that this form of the species may be

more or less closely related to Rosenbaum's new strain, as shown by contrast with his data. Unfortunately no direct comparison could be made with his material.

ROSENBAUM'S NEW STRAIN	P1 OR H
(1) More pathogenic on the stems of potato than other strains or isolations from that host; able to produce a distinct necrosis of the tissues of the potato tuber.	(1) The strains are more pathogenic on stem of potato, lettuce, egg-plant, and bean than the remaining strains.
(2) On potato agar this strain produces in 7–10 days a marked discoloration (dark brown to black) of the medium; while other strains, if they produce color at all, never approach the intensity produced by this strain.	(2) On potato agar, these strains produce a blackish discoloration, by which means the strains may be easily distinguished from the others studied.
(3) On corn-meal agar, there are produced light gray, loosely formed sclerotia, as compared with the darker, brownish, and more compact sclerotia of other strains.	(3) The characteristics referred to by Rosenbaum are not only observed on corn meal agar, but also on potato agar, bean agar, rice meal agar, and other media.
(4) On Uschinsky's solution, after 10 days, the strain covered the surface and was growing on the side of the flask, while in the other growth was still submerged.	(4) Unfortunately these strains were not cultured on Uschinsky's solution, but the tendency observed by Rosenbaum is strikingly noticed on all liquid media used.
(5) The diameter of the hyphae varies from 4.7 to 8.8 μ, with 7.8 μ as the average measurement.	(5) The diameter of the hyphae of these varies from 7 to 12 μ, with 9 μ as the average measurement.
(6) Sclerotia: cells measure in length 13.6–30.6 μ, averaging 21.6 μ; in width, 8.3–20.4 μ, with an average of 12.3 μ.	(6) Sclerotia: cells measure in length 17–59 μ, averaging 38 μ; in width, 8–20 μ.

It is thus shown that with the exception of minor differences in the dimensions of sclerotia the characteristics presented are concordant. Again, it may also be inferred that Rosenbaum's "other strain" may be identical with either B1 or P4.

Concerning the relationship between P1 and P4 there are marked differences in morphological and physiological characteristics, as shown by dimensions and color of sclerotia, by diastase and invertase activities, by temperature requirement, by cultural characters, and by pathogenicity. Nevertheless, these differences may not perhaps be considered sufficient to distinguish these permanently as different species, especially since these characteristics are more or less modified by environment, particularly by a change of host plants. Some important indications obtained by a study of reisolated cultures of these two strains, made from sclerotia appearing on potato tubers artificially inoculated (see inoculation experiment no. 5), are shown below.

REISOLATED STRAIN P1

(1) Sclerotia: cells 16–23 ×24–48 μ, dark brown in color.

(2) Hyphae: diameter 8–14 μ, turning dark brown when old.

(3) Pathogenicity: not so virulent as the original P1.

(4) Cultural characters: blackening of potato agar is not so striking as that of original strain.

(5) Diastatic efficiency: 92 (original strain averages 45).

REISOLATED STRAIN P4

(1) Sclerotia: cells 16–22 ×23–48 μ, dark brown in color.

(2) Hyphae: diameter 8–14 μ, turning dark brown when old.

(3) Pathogenicity: about the same as the original P4 or reisolated P1.

(4) Cultural characters: blackening of agar is about the same as in the original strain P4.

(5) Diastatic efficiency: 100.

The evidence presented above makes it safe to assume that these two strains P1 and P4 may be properly regarded as a single species modified more or less by environment. In general, the strain P4, as Duggar ('16) says, in itself scarcely merits consideration as a causal fungus of disease and I find it less virulent than the strain P1. As a matter of fact, in no case may the strain P4 be so changed as to resemble the form P1, either by changing the culture media or host plants, while P1 may be easily transformed into P4 as has been shown.

The strain B1 is notably distinguished from the remaining strains by its characteristic sclerotia, by its cultural characters, and by having a higher temperature requirement for mycelial

growth, etc. Nevertheless, these characteristics alone are not considered of specific importance on account of indications given later. P1 and B1 were reisolated from affected pods of navy beans used for an inoculation experiment (see inoculation experiment No. 7). Morphological and physiological characteristics of these two reisolated strains may be compared as follows:

REISOLATED STRAIN P1

(1) Sclerotia: cells 12–19 ×22–56 μ.

(2) Hyphae: diameter 8–14 μ, turning dark brown when old.

(3) Invertase activity: (similar to reisolated B1).

(4) Temperature requirement: optimum temperature is about the same as that of the original strain P1.

REISOLATED STRAIN B1

(1) Sclerotia: cells 14–21 ×20–50 μ.

(2) Hyphae: diameter 8–15 μ, turning dark brown when old.

(3) Invertase activity: (similar to reisolated P1).

(4) Temperature requirement: optimum temperature is somewhat lower, as compared with that of the original strain B1 (28° C.).

The characteristics and behavior of these two reisolated strains are rather similar to those of the original strain P1, although the pathogenicity of the reisolated strain B1 is not so pronounced as that of P1. The strains compared may be regarded as two physiological forms of *Rhizoctonia Solani* Kühn.

P7 is strikingly distinguished from the remaining strains by its morphological and physiological characters, and is considered to be new to science. This strain, however, does not seem to be particularly virulent. So far as my present inoculation experiments are concerned, this strain may be responsible for some secondary infections. No definite conclusion may be drawn at present concerning the taxonomic relations of B3, owing to lack of sufficient data.

Summary

1. From the macroscopical and microscopical investigation of the 15 different isolations of *Rhizoctonia* obtained from a wide range of hosts of different geographical origin, it was possible at the outset to identify some conclusively, so that the number was reduced to 6 different types for further physiological studies, namely, P1, P4, P7, B1, H, and B3.

2. The temperature requirements of P1 and H are similar, while the remaining strains exhibit different optima, minima, and maxima.

3. All the strains hydrolyze starch, but the diastatic activity is unlike. The activity of P4 is notable and stands above that of the others. B1 has the next higher capacity, and P1 and P7 the minimum.

4. All these fungi are able to convert cane sugar. This inverting activity of P4, P7, and B1 is striking and many times that of P1.

5. Maltase and lactase activity was qualitatively and quantitatively determined, but insufficient data have been collected to determine the extent of specialization. The nutritive value of lactose is markedly less than that of maltose.

6. All the fungi are unable to utilize inulin.

7. Glucose, fructose, and galactose are utilized by these fungi. The availability of these sugars is about the same, and no marked specialization was noticed in any of the strains.

8. Amygdalin is utilized as a source of carbon, being, of course, first decomposed by emulsin before becoming available. No marked difference in emulsin activity was observed in any of the strains studied.

9. Cellulase is present in the mycelium of these strains. Its activity was not measured quantitatively, but qualitative studies indicate that it is highest in P1 and H.

10. P1 and H grow best on casein, peptone, and asparagin as sources of nitrogen and carbon, and less well on legumin, while P7 grows best on peptone, legumin, casein, and asparagin, in the order named. P4 grows best, and equally well on peptone and casein, and it grows less on legumin and asparagin.

11. Potassium nitrate, ammonium sulphate, and ammonium nitrate are available as sources of nitrogen, though potassium nitrate is preferable.

12. P1 and H utilize potassium nitrite, while the remaining strains cannot do so. Reduction of nitrate is observed in the potassium nitrate culture by P1 and H, also P4 and B1. Absolutely no reduction takes place in P7.

13. As a whole, the mycelial growth is more sensitive to modification in the carbohydrate supply than to changes in the nitrogen supply.

14. The growth relations of B1 on liquid media were changed after successive transfers on artificial culture media.

15. No definite cultural characterization of B3 is possible from the results thus far obtained.

16. The presence of trypsin and erepsin was observed in the mycelium of all the strains studied.

17. Examined as to hydrogen-ion concentration all strains show increased growth on the acid side of neutrality. In general, all yield well on media with an exponent somewhat larger than P_H 3.8. P4 and P7 exhibit a somewhat narrower range on the acid side, while B1 has the widest acid range.

18. In these fungi the general tendency is to increase the active acidity during growth, and this increase seems to be proportional to the increase of growth.

19. Fusion occurs between hyphae arising from the different mycelia of the same strain. Fusion is also observed when P1 and H are sown in a drop culture, also between P1 and P4, although this is not so frequent as in the case of P1 and H. No fusion occurs between B1 or P7 and any one of the others. From the experiments it is inferred that fusion may take place only between the hyphae from strains which are rather closely related, or which have rather recently originated from the same ancestral type.

20. The effect of inadequate aëration is to repress the growth of mycelium and sclerotial formation in all strains.

21. From the inoculation experiments it is concluded that P1 and H may attack all the plants studied, generally causing "damping off." B1 also attacks certain hosts, but it is less virulent than the others mentioned. The virulence of P4 is slight. In no case has direct infection by P7 and B3 been observed.

22. The pathogenicity of the strain P1 was more or less modified by a transfer to a host plant different from that on which it was originally found, but the highest pathogenic capacity of the strain is always manifest when inoculation is made on plants belonging to the same species of host as that from which the culture originated.

23. The hyphae of these fungi may enter the host tissue directly through the cuticle, and the penetration of such hyphae is chiefly a mechanical process.

24. Infection of the host takes place much more easily through the root than through any other part.

25. P1 and H are identical in morphological and physiological characteristics and constitute a single form of *Rhizoctonia Solani* Kühn. This form is a very common type of the species, causing serious diseases of many cultivated plants. It may be identical with Rosenbaum's "new" strain.

26. While P1 and P7 might have been derived from the same ancestral origin, some striking physiological specializations have been developed.

27. It is desirable to regard B1 as a physiological strain of *Rhizoctonia Solani* Kühn rather than as a distinct species.

28. P4 and B1 may be two distinctly specialized forms of *R. Solani* Kühn but cannot be considered distinct species.

29. P7 is distinct from all other strains studied, and the differences manifested by this strain may be sufficient to be considered of specific rank.

The author wishes to express here his heartiest thanks to Dr. B. M. Duggar, under whom this work was prosecuted, for many valuable suggestions. Thanks are also due to Dr. George T. Moore for the privileges of the laboratory and library.

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